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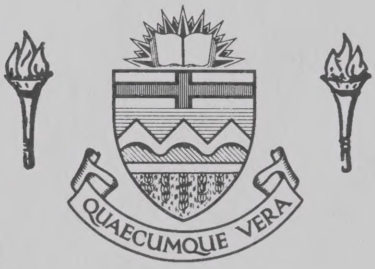

THE UNIVERSITY OF ALBERTA

EFFECTS OF DIETARY PROPIONATE ON LIPID METABOLISM IN GROWING SWINE.

by

© PHILIP ALFRED THACKER

A THESIS
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ABSTRACT

Two experiments were conducted utilizing 144 crossbred pigs to determine the effects of dietary propionic acid (PA) or calcium propionate (CP) on lipid metabolism in swine. In the first experiment, isonitrogenous and isoenergetic diets, based on either barley-soybean meal or barley-canola meal were fed supplemented with either 0, 3, 6 or 9% PA. Inclusion of PA reduced average daily feed (ADF) intake and average daily gain (ADG) but did not significantly influence feed conversion efficiency (FCE). Dietary inclusion of PA significantly reduced backfat thickness and significantly increased levels of 15:0, 17:0 and 17:1 fatty acids in the outer subcutaneous backfat layers with an accompanying decrease in 16:0 and 18:0 fatty acids. Inclusion of PA significantly reduced the levels of total serum cholesterol, associated entirely with a reduction in the high density lipoprotein (HDL) fraction. There was a slight increase in the lipid content of both hearts and livers due to dietary PA.

Pigs fed diets supplemented with 15.9 to 18.9% canola meal had reduced ADF, ADG, FCE and reduced total backfat compared with those fed diets supplemented with soybean meal. Pigs fed diets supplemented with soybean meal had lower levels of total serum cholesterol, low density lipoprotein (LDL) cholesterol and serum triglycerides compared with those fed canola meal.

In the second experiment, diets based on barley-soybean meal, containing 0, 3.5 or 7% PA or CP were fed with and without the addition of 4.95 mg vitamin B-12/kg diet. The addition of PA depressed ADF, while CP had no effect. ADG was reduced when either 7% PA or CP was fed, but ADG was slightly improved when 3.5% PA or CP was fed. FCE was improved when 3.5 or 7% PA or 3.5% CP was fed but 7% CP depressed FCE. Vitamin B-12 supplementation slightly improved the ADG and FCE of pigs fed CP but had no effect on pigs fed PA. Addition of CP significantly reduced the thickness of carcass backfat in treated pigs while supplementation with vitamin B-12 eliminated this reduction. A similar though non-significant trend was observed with PA. Pigs fed 7 % PA or CP had significantly higher levels of FA 17:0 and 17:1 in carcass backfat. Addition of vitamin B-12 reduced this effect.

Addition of PA or CP depressed serum cholesterol, HDL cholesterol, LDL cholesterol and serum triglycerides. Vitamin B-12 supplementation had no effect on serum total cholesterol or HDL cholesterol, caused a reduction in LDL cholesterol and tended to overcome the reduction in serum triglyceride. Supplementation of diets with vitamin B-12 reduced liver lipid levels largely as a result of a decrease in cholesterol and phospholipids.

The overall results suggest that dietary propionate will reduce the backfat thickness of treated pigs. The results suggest that the reduction in backfat thickness is

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not strictly due to a reduction in feed intake but may be the result of accumulation of methylmalonyl-CoA and its effects on fat synthesis. Propionate may also be effective in reducing serum cholesterol levels. The lower levels of HDL cholesterol suggest that one mechanism by which propionate reduces total serum cholesterol is through a shift in body cholesterol from serum to the tissue pool.

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I. INTRODUCTION

Coronary Heart Disease is the major cause of death in the developed world today, being responsible for almost 50% of all human mortality. Mortality from Coronary Heart Disease far exceeds that from diseases such as tuberculosis, diphtheria, small pox or cancer (Keys 1970). It is generally agreed that the underlying cause of most cardiovascular disease is the development of atherosclerosis.

Atherosclerosis is principally a disease of the large arteries in which lipid deposits appear in the subintimal layer of the arteries (Gurr and James 1971). These plaques contain an especially large amount of cholesterol and are usually associated with degenerative changes in the arterial wall. As the disease progresses, fibroblasts infiltrate the degenerative areas and cause progressive sclerosis of the arteries. In addition, calcium often precipitates with the lipids to develop calcified plaques. When these two reactions occur the arteries become extremely hard. Atherosclerotic arteries lose most of their distensibililty and because of the degenerative areas, they are easily ruptured. The atheromatous plaques often protrude through the intima into the flowing blood, and the roughness of their surfaces causes blood clots to develop which may result in embolism formation and possibly completely occlude the blood vessel.

Nearly three decades of intensive epidemiological investigations have identified a group of characteristics

that predict an individual's probability of developing coronary heart disease. These characterisitics are known as "risk factors", a descriptive but non-committal term which avoids the question of whether these characterisitics are causative agents, intervening variables, early manifestations of disease, or secondary indicators of an underlying disturbance. The most commonly identified "risk factors" are heredity, stress, obesity, hypertension, diabetes mellitus, cigarette smoking, lack of exercise, diet and hypercholesterolemia (Strong and Eggen 1970).

The theory that circulating cholesterol is a key factor in the development of atherosclerosis is supported by a large body of evidence. This evidence has been derived from experimental, clinical and epidemiological studies.

Studies in different human populations have shown that those with high concentrations of plasma cholesterol have a higher incidence of atherosclerosis than those with low plasma cholesterol (Kannel et al. 1979; Miller et al. 1977). Men with plasma cholesterol levels below 190 mg per 100 ml are reported to be at only half the risk for coronary heart disease as those with levels of 190 to 250 mg per 100 ml (Grundy 1978).

The consistent observation that human atherosclerosis is prominent in clinical states accompanied by hypercholesterolemia have further implicated cholesterol as an important etiological agent in the development of atherosclerosis. Diseases such as diabetes mellitus,

nephrotic syndrome, hypothyroidism, and familial hypercholesterolemia are all accompanied by elevated cholesterol levels and atherosclerosis (Katz and Stamler 1953).

Serum cholesterol concentration can be elevated in many animal species by feeding a high fat, high cholesterol diet resembling those consumed by humans and these animals develop intimal lipid deposits resembling atherosclerotic streaks (Ratcliffe et al. 1970). Therefore the relationship between an elevated plasma cholesterol and atherosclerosis seems well established.

Numerous chemicals have been tested for their effectiveness in reducing serum cholesterol (Sabine 1977). These chemicals may function by inhibiting cholesterol synthesis, blocking cholesterol absorption or enhancing cholesterol excretion. It is hoped that a reduction in serum cholesterol, through drug intervention, may result in a reduction in the rates of cholesterol deposition in the arterial wall and therefore reduce the risk of development of Coronary Heart Disease. Dietary propionic acid may be effective in reducing serum cholesterol levels as it inhibits the activity of the enzyme β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase, which catalyzes the rate limiting step in cholesterol synthesis (Rodwell et al. 1976).

Several hypotheses have been put forward in an effort to explain the high prevalence of Coronary Heart Disease in

western populations. Of these, that implicating the quantity and quality of dietary fat is the most widely acepted (Glueck 1979). Other hypotheses implicating sugar intake (Ahrens 1974), water hardness (Anon 1967), fiber intake (Trowell 1972) and levels of dietary copper and zinc (Klevay 1975) have received less acceptance.

As a result of evidence linking fat-rich diets to high serum cholesterol, and high serum cholesterol levels to coronary heart disease, the American Medical Association has recommended that persons falling in the "high risk" categories, substantially decrease their intake of saturated fat and lower their cholesterol consumption (Livingston 1974). As a consequence, consumers are discriminating against animal fat. Therefore the development of methods to control fat accumulation in the domestic pig has become one of the foremost goals of the hog industry.

Research with swine falls into two categories: research directed towards efficient production of pork to supply human food and research directed towards gaining basic knowledge of human health (Pond and Houpt 1978). In studies reported in this thesis, an effort was made to utilize the pig as a test animal to meet both criteria. A series of experiments was conducted to determine the effects of dietary propionic acid or propionate on levels of serum cholesterol and on backfat thickness in growing pigs, and to determine possible mechanisms for the mode of action of propionate.

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II. CHOLESTEROL METABOLISM

A. Absorption

Although an elevated serum cholesterol has been implicated as a risk factor in the development of atherosclerosis, it is important to realize that the body cannot function without cholesterol. Less than 7% of the cholesterol in the body is actually found in the plasma (Sodhi et al. 1979). The remainder is distributed in the tissues and plays a vital role in the body's metabolism. Cholesterol is essential for the structural and functional integrity of all cellular and intracellular membranes in the body. It is a precursor in the synthesis of bile salts and steroid hormones and is vital in the transport system of fatty acids.

The absolute amount of cholesterol contained within the tissues of the body at any one time is the net balance at that time between dietary inputs and body synthesis on one hand, and excretion of neutral steroids and oxidation to bile acids on the other (Naber 1976)

Intake of cholesterol in developed countries is approximately 500-750 mg per day (Grundy 1978). This intake is derived entirely from animal products such as meat, eggs, milk and butter. In addition, approximately 750-1250 mg of cholesterol per day enters the intestinal tract in the form of bile acids. Although the cholesterol in bile is unesterified, a small fraction in the diet may be esterified

with long chain fatty acids. However, any cholesterol ester entering the intestine is hydrolyzed by pancreatic cholesterol esterase and it is the free form of cholesterol which is absorbed (Grundy 1978).

In order to be absorbed, cholesterol must be in a soluble state (Grundy 1978). Solubility is facilitated by encasing the cholesterol with bile salts, phospholipids and other lipids in mixed lipid micelles. Because the micelles are water soluble, they allow cholesterol to be transported through the aqueous environment of the intestinal lumen to the brush border of the mucosal cells. The micelle as a whole does not penetrate the mucosal cell membrane, but releases its cholesterol to the cells of the intestinal wall, where it enters by a passive diffusion process. The primary site of cholesterol absorption is the proximal small intestine, primarily the duodenum and jejunum (Grundy 1978).

Once inside the mucosal cell, the absorbed cholesterol mixes with the endogenous cholesterol present in the cell and 80-90% is esterified by an enzyme similar to or identical with pancreatic cholesterol ester hydrolase (Sabine 1977). This cholesterol ester is transported away from the intestine in the lymph as part of both chylomicrons and Very Low Density Lipoproteins (VLDL) (Grundy 1978).

Bile salts are obligatory for cholesterol absorption and when they are diverted from the intestine, as in total bilary obstruction, no cholesterol is absorbed (Siperstein et al. 1952). The rate of cholesterol absorption increases

as dietary fat increases, possibly reflecting the necessity for polar lipids in maintaining cholesterol solubility in bile salt solutions. Varying the level of dietary cholesterol will also affect the amount absorbed (Grundy 1978). Another group of dietary constituents affecting cholesterol absorption are the plant sterols. These sterols, of which β-sitosterol is the most common, differ only slightly in chemical structure. They interfere with cholesterol absorption, but for some unexplained reason are themselves absorbed in only trace amounts (Grundy 1978).

B. Cholesterol Synthesis

Endogenous synthesis of cholesterol appears to be adequate for the normal growth and health of animals and therefore it is not an essential ingredient in the human diet.

The first step in cholesterol synthesis involves the activation of acetate by coenzyme A, following which, two molecules of acetyl-CoA condense to form acetoacetyl-CoA in a reaction catalyzed by an enzyme called β-ketothiolase (Sodhi et al. 1979). Acetoacetyl-CoA serves as an intermediate for synthesis of fatty acids and ketone bodies as well as cholesterol. Acetoacetyl-CoA condenses with another molecule of acetyl-CoA to form HMG-CoA catalyzed by HMG-CoA synthase. This compound can either be converted to ketone bodies or to cholesterol. The conversion of HMG-CoA to mevalonic acid by HMG-CoA reductase is irreversible and

is the rate limiting step in cholesterol synthesis (Rodwell et al. 1976). Mevalonate is converted through a series of steps to form farnesyl pyrophosphate and squalene. Squalene is an open-chain hydrocarbon which upon cyclization is converted to lanosterol which can be demethylated and reduced to form cholesterol.

With the exception of the adult brain, all cells are capable of cholesterol synthesis (Sodhi et al. 1979). However, the rates of cholesterol synthesis differ markedly from one tissue to another. The most active organ of cholesterol synthesis is the liver (Sodhi et al. 1979). Experimental data from rats and monkeys, indicated that of the total cholesterol synthesized in the body, 80% is produced in the liver, about 10% in the gastrointestinal tract, and about 5% in the skin. The remainder is synthesized in all other body tissues combined. However, in the pig, it appears that adipose tissue and the gastrointestinal tract are more important sites of cholesterol synthesis than is the liver (Huang and Kummerow 1976).

Cholesterol synthesis is inhibited by the presence of cholesterol in a negative feedback fashion (Tompkins et al. 1953). Dietary cholesterol will inhibit hepatic synthesis of cholesterol but does not appear to affect synthesis in the intestine.

Rates of hepatic cholesterolgenesis may depend on several factors besides feedback regulation by cholesterol.

One of these factors may be bile acids. The role of bile acids in the regulation of hepatic synthesis however is controversial and complex. The bile acids may influence cholesterol synthesis in at least three ways. First, since bile acids affect cholesterol absorption, an increase of bile acids in the intestine should promote absorption, which in turn will suppress cholesterol synthesis. Second, bile acids suppress their own synthesis from cholesterol and should thereby increase hepatic concentration of cholesterol. This second mechanism should reduce synthesis through feedback inhibition by cholesterol itself. Third, bile acids may directly interfere with some step in cholesterol synthesis (Grundy 1978).

Synthesis of cholesterol may depend to some extent on food intake. Production rates during feeding greatly exceed those of fasting which may reflect availability of dietary substrate. Chronically high intakes of energy are associated with an overproduction of cholesterol, while a restriction of energy reduces synthesis (Grundy 1978).

C. Cholesterol Transport

Since cholesterol is insoluble in aqueous solution, it cannot circulate freely in plasma and must be brought into solution by more complex mechanisms, specifically by lipoproteins. Lipoproteins are spherical particles composed of a liquid core of non-polar lipids (triglycerides and cholesterol esters) covered by a mono-molecular film of

polar lipids primarily phospholipids. The surface also contains unesterified cholesterol in relatively small amounts together with proteins called apoproteins. Through interactions with enzymes and cell surface receptors, the apoproteins direct each lipoprotein to its site of metabolism (Brown et al. 1981).

Based on ultracentrifugational and electrophoretic studies, there are four major classes of lipoproteins; chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Triglyceride content decreases and cholesterol content increases as density increases (Kannel et al. 1979).

The apoprotein composition of lipoproteins also varies among lipoprotein classes. For example, apoprotein B is the major protein of LDL and is present in VLDL. Apoprotein C is a major apoprotein of VLDL and is regularly found in HDL and in minor amounts in LDL. Apoproteins A-1 and A-11 are the major proteins of HDL (Smith et al. 1978).

The largest lipoprotein in plasma is the chylomicron, which is produced by the intestine in response to a fatty meal (Grundy 1978). The core of the chylomicron is rich in triglycerides which are assembled in the intestinal mucosa from dietary fat. The core also contains small amounts of cholesterol esters derived from newly absorbed cholesterol. The intestine incorporates these lipids into chylomicrons which are secreted into the lymph and from there enter the blood stream. The chylomicrons are too large to cross the

endothelial barrier and are therefore metabolized while they are still in the blood stream. For this purpose the chylomicrons bind to lipoprotein lipase, an enzyme that is fixed to the luminal surface of endothelial cells that line capillaries of adipose and muscle tissues (Brown et al. 1981). The chylomicrons contain an apoprotein that activates the lipase and liberates free fatty acids and monoglycerides. The fatty acids enter the adjacent muscle or adipose cells where they are either oxidized or reesterified for storage. As the trigyceride core is depleted, the chylomicron shrinks. The excess surface material, primarily phospholipids and free cholesterol, is transferred to another plasma lipoprotein HDL. The depleted chylomicron is released from the capillary wall and reenters the circulation. The particle known as a chylomicron remnant retains its cholesterol ester and apoprotein B. The remnant is carried to the liver where it binds to receptors on the surface of hepatic cells. The remnants are immediately brought into the cell by receptor-mediated endocytosis and degraded in lysosomes (Brown et al. 1981).

The liver, like the gut, secretes a triglyceride-rich lipoprotein, the VLDL. This particle has a structure similar to chylomicrons except that it contains more cholesterol and has a smaller size. When dietary cholestrol is available, the liver uses that source of sterol, derived from receptor-mediated uptake of chylomicron remnants, for lipoprotein synthesis. When dietary cholesterol is

insufficient, the liver synthesizes its own cholesterol by increasing the activity of the rate-controlling enzyme HMG-CoA reductase (Brown et al. 1981).

The VLDL particles interact with lipoprotein lipase in capillaries releasing most of the triglycerides (Brown et al. 1981). As the size of the VLDL particle diminishes, owing to its interaction with lipoprotein lipase, the density increases and the particles are converted to intermediate density lipoproteins (IDL). The excess surface materials, mostly phospholipids and cholesterol are transferred to HDL. The HDL particles interact with Lecithin:Cholesterol Acyltransferase (LCAT) which esterifies the cholesterol with fatty acids derived from the 2 position of lecithin, the major phospholpid of plasma. The newly synthesized cholesterol ester is transferred back to IDL through the action of a plasma cholesterol ester exchange protein. The net result of the coupled lipolysis and exchange reactions is the replacement of most of the triglyceride core of VLDL with cholesterol esters (Brown et al. 1981).

After lipolysis, the IDL particles are released from the capillary wall into the circulation. They then undergo a further conversion in which most of the remaining triglycerides are removed and all of the apoproteins except apoprotein B are lost. The resultant particle, which contains almost pure cholesterol ester in the core and apoprotein B at the surface is LDL. The catabolism of the

LDL is beleived to occur within the peripheral cells (Goldstein and Brown 1977).

Uptake of LDL by peripheral cells appears to be mediated by LDL-binding receptors on the surface of the cells. The extent of the binding is a function of the number of receptors, which is regulated in turn by the cells need for cholesterol. If a cell contains an abundance of cholesterol, the production of receptors is reduced, thereby decreasing the cellular uptake of cholesterol. In contrast, depletion of cellular cholesterol increases the number of receptors and uptake is facilitated (Grundy 1978).

The LDL binds to these high affinity receptors located in the region of the plasma membrane, called coated pits. These pits invaginate into the cell and pinch off to form endocytic vesicles that carry the LDL to lysosomes (Brown et al. 1981). Fusion of the vesicle membrane with the lysosomal membrane exposes the LDL to hydrolytic enzymes that degrade the apoprotein B to amino acids. The cholesterol esters are hydrolyzed by an acid lipase and the liberated cholesterol is discharged into the cell cytoplasm where it can be incorporated into cell membranes, reesterified for storage within the cell, or excreted out of the cell. The increased intracellular cholesterol concentration results in a decrease in the activity of HMG-CoA reductase, thus reducing the intracellular synthesis of cholesterol. There is an increase in the activity of cholesterol acyltransferase, thus stimulating the reesterification of cholesterol, and

also a decrease in the number of LDL binding sites, thereby reducing the further uptake of cholesterol. As a result of this LDL uptake mechanism, extrahepatic cells have low rates of cholesterol synthesis relying instead on cholesterol derived from LDL (Brown et al. 1981).

If saturation of the receptor occurs, as may occur at high plasma LDL levels, LDL may be taken up by a phagocytic, receptor independent process (Goldstein and Brown, 1977). After phagocytosis, cholesterol esters are hydrolyzed, but free cholesterol acquired in this way does not inhibit HMG-CoA reductase or stimulate esterification, leading to uncontrolled accumulation of cholesterol (Grundy 1978). An elevated LDL is therefore one of the prime factors in the development of atherosclerosis (Scanu 1978; Goldstein and Brown 1977).

In contrast, an elevated HDL appears to exhibit a protective effect towards the development of atherosclerosis (Havel 1979; Grundy 1979). There is an inverse relationship between the levels of plasma HDL and the incidence of coronary heart disease (Miller and Miller 1975; Castelli et al. 1977)

The HDL are secreted primarily in the liver and enter the circulation as relatively empty biconcave sacs, consisting mainly of protein and phospholipid, but with little cholesterol (Witztum and Schonfeld 1979). It is speculated that HDL pick up cholesterol from peripheral cells and transport it to the liver where it may be excreted

as cholesterol or bile acids (Havel 1979). The relatively small size of the HDL would seem to suit it for the role of accepting cholesterol from peripheral tissues as the particle is able to pass rapidly into capillaries and into interstitial fluid where it comes into contact with cholesterol-rich cell membranes (Grundy 1978). The HDL pick up cholesterol from the cells and an enzyme Lecithin:Cholesterol Acyltransferase transfers a fatty acid group from the phospholipid lecithin to the cholesterol to form cholesterol ester. This material, which is highly nonpolar and hydrophobic, moves to the interior of the HDL particle where it is sheltered from the aqueous environment of the blood by the polar proteins and phospholipids on the polar surface. The HDL continues to pick up cholesterol and gradually becomes sperical. The filled HDL particles return to the liver where the cholesterol is metabolized. The tissue pool of cholesterol has been shown to be inversely related to the concentration of HDL (Miller et al. 1976). By removing cholesterol from peripheral tissues, including the arterial wall, the HDL may function in retarding the development of atherosclerosis (Scanu 1978).

A further explanation for the protective effects of the HDL has been proposed by Carew et al. (1976), who studied the surface binding, internalization and degradation of radioiodinated lipoproteins by smooth muscle cells. When I^{125} labelled LDL was incubated with cells in the presence of increasing concentrations of HDL, the binding,

internalization, and degradation of the LDL were all decreased. It is suggested that the HDL compete for receptor sites on smooth muscle cells, but enter the cell and are degraded at a much slower rate. The reduction in LDL uptake is postulated to slow down the atherosclerotic process.

The importance of HDL is revealed in patients with the rare familial disorder called Tangier's disease. In this disease, HDL levels are extremely low. These patients demonstrate marked accumulations of cholesterol in many tissues of the body, including spleen, lymph nodes, intestinal mucosa and blood vessels and develop atherosclerosis at a very early age (Grundy 1979).

In several large epidemiological studies, such as the Framingham Study, coronary heart disease has been less common in people who have higher levels of HDL (Gordon et al. 1977).

Studies have shown that women have higher average levels of HDL than do men; and since women are less prone to atherosclerocis than men, the higher HDL could be one factor in this protection (Levy et al. 1966). Experimental studies in women have shown that estrogens cause an increae in HDL concentration (Cheung and Albers 1977), thus the estrogenic hormones in women may be the cause of the relatively high HDL.

Family members with familial hypercholesterolemia (increased LDL) have shortened life expectancies and high levels of coronary risk, whereas subjects from families with

familial hyperalphalipoproteinemia (increased HDL) have increased longevity and lower than usual incidence of coronary risk (Glueck et al. 1975).

Animals with low levels of LDL and or high levels of HDL such as the dog and rat, manifest little spontaneous or experimentally induced atherosclerosis whereas swine and some species of monkeys whose lipoprotein distibutions more nearly reflect those of man, manifest spontaneous atherosclerosis and vascular lesions can be produced experimentally (Witztum and Schonfeld 1979). In certain marine animals, elevations in serum cholesterol of up to 500 mg/100 ml may be found with no atherosclerosis. These animals carry most of their cholesterol in the HDL (Scanu 1978).

D. Cholesterol Excretion

Cholesterol, along with its major bacterial metabolites, coprostanol and coprostanone, is lost in the feces at a rate estimated at between 1-3 g/day. A further loss, estimated to be in the order of 200-600 mg per day, occurs in the bile (Sodhi et al. 1979). The liver converts cholesterol into cholic acid and chenodeoxycholic acid. These are excreted in the bile but are largely reabsorbed in the lower ileum by an active process (Grundy 1978). The bile acids which escape reabsorption are deconjugated by the bacterial flora in the large intestine and are further degraded to so called secondary and tertiary bile acids. The

major microbial product of deconjugated cholic acid is deoxycholic acid and that of chenodeoxycholic acid is lithocholic acid. A further loss estimated at less than 10% of the daily total production occurs in the urine. This is the result of the loss of the metabolites of adrenal and sex hormones which were originally synthesized from cholesterol.

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III. PROPIONIC ACID METABOLISM

Propionic acid is a short chain volatile fatty acid usually thought of as a by-product of microbial fermentation in the rumen. However, it is becoming increasingly evident that swine also possess areas of their gastrointestinal tract where microbial fermentation occurs and that volatile fatty acids play a role in contributing to the maintenance energy requirements of these animals (Kennelly et al. 1981; Imoto and Namioka 1978; Kass et al. 1980). Propionic acid may also arise from β-oxidation of odd-numbered fatty acids and from the degradation of branched aliphatic amino acids (Kaziro and Ochoa 1964).

In mammalian systems, propionic acid has only one route of metabolism. It is first activated with ATP and coenzyme A to form propionyl-CoA. The activated propionate is then carboxylated to form methylmalonyl-CoA and finally isomerized under the influence of methylmalonyl-CoA isomerase to succinyl-CoA, a TCA cycle intermediate. This last step requires vitamin B-12 as a cofactor and when levels of vitamin B-12 are inadequate methylmalonyl-CoA will accumulate in the body tissues (Frenkel et al. 1974).

There is little propionic acid present in normal feedstuffs used in swine rations. However, large quantities are available as by-products of the chemical industry. Bowland et al. (1971) reported that feeding up to 8% of a mixture of volatile fatty acids had no deleterious effects on the performance of growing pigs.

Propionic acid has been utilized in the preservation of high moisture grain to decrease microbial activity when the grain is stored exposed to air. Several studies have been conducted utilizing these acid treated grains in swine diets. Trials have been conducted utilizing acid-treated corn (Young et al. 1970; Jones et al. 1970; Forsyth 1975), barley (Perez-Aleman et al. 1971; English et al. 1973; Cole et al. 1980) and rapeseed (Bowland 1972; Bowland and Newell 1974). In general, performance of pigs fed acid-treated grains was as good as or better than that of pigs fed the dried grain.

Work done some time ago by Wood and Migicovsky (1956) indicated that the addition of odd chain fatty acids to cholesterol synthesizing systems inhibited cholesterol synthesis. More recently Bush and Milligan (1971) reported that the addition of propionate or propionyl-CoA to bovine liver homogenates inhibited the activity of the enzyme HMG-CoA synthase. The conversion of HMG-CoA to mevalonic acid is the rate limiting step in cholesterol synthesis (Rodwell et al. 1976) and therefore feeding of propionic acid may be effective in reducing serum cholesterol levels.

Preliminary studies by Salomons (1977) indicated that dietary propionic acid was effective in reducing the serum cholesterol level of treated pigs. However, Boila et al. (1981) were not able to confirm this observation.

Methylmalonyl-CoA, a breakdown product of propionic acid metabolism, has been shown to cause a reduction in

fatty acid synthesis (Cardinale et al. 1970). The synthesis de novo of fatty acids from acetyl-CoA is catalyzed by acetyl CoA carboxylase, which catalyzes the conversion of malonyl-CoA from acetyl CoA, and fatty acid synthetase which catalyzes the synthesis of long chain fatty acids from malonyl-CoA. The activity of both these enzymes has been shown to be markedly inhibited by the presence of methylmalonyl-CoA (Frenkel et al. 1973).

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IV. ANIMAL MODELS IN MEDICAL RESEARCH

Animal models are an invaluable tool in medical research and have done much to further the knowledge and understanding of human health and disease. Researchers have studied the animal in order to extrapolate to man. They have recognized in the experimental animal an analogy of man and have modified the animal to become a model of man or of diseased man. The ultimate goal has been the application of the results obtained from animals in order to improve the welfare of man.

The choice of an experimental model for medical research studies has usually been confined to rodents, rabbits, cats and dogs. Convenience has generally played a major role in the final choice of species. Recently, greater attention has been paid to the importance of selection of animals on the basis of similarity to man, or of particular features pertinent to a study. The utilization of swine and particularly miniature swine in biomedical research has increased substantially in recent years (Pond and Houpt 1978). This increased application has resulted largely from an awareness that man and swine have many biological similarities and therefore, experimental data obtained with swine may be extrapolated to man.

There are many advantages to the utilization of swine as an experimental animal. The genetic pool in swine is diverse, the reproductive cycle short and the prolificacy high so that animals can be chosen to fit a wide variety of

research purposes (Pond and Houpt 1978). Veterinary science already has a substantial body of information about pigs. Consequently, the use of the pig in a study would not be handicapped by a lack of knowledge about the basic anatomy, physiology or pathology of the species. The larger size of swine in relation to many common laboratory animals facilitates the acquisition of larger amounts of tissue or fluids. Also their relatively long life span is important in relation to their use in radiation studies or gerontological research. Finally the relative cost of swine is considerably less than that of some other common laboratory animals such as the dog or primate. However, all this would be for nought if there were not so many anatomical similarities between swine and man.

The pig is similar to the human in dental characteristics (Weaver and McKean, 1965), renal morphology and physiology (Nielson et al 1966), eye structure and visual acuity (Prince et al. 1960), skin morphology and physiology (Montagna 1966), cardiovascular anatomy and physiology (Luginbuhl 1966) and digestive anatomy and physiology (Book and Bustad 1974).

As a result of these biological similarities between swine and man, numerous publications have elaborated on the potential of swine as an ideal experimental animal (Book and Bustad 1974; Bustad 1966; Bustad and McClellan 1965; Douglas 1972; Mount and Ingram 1971; Glauser 1966). In addition, a symposium devoted to an analysis of uses of the pig in

biomedical research has highlighted important contributions of swine to medical research (Bustad et al. 1966)

The pig is one of the most suitable models for experimental study of human atherosclerosis because it is susceptible to atherosclerosis under normal conditions of husbandry, is readily available at resonable cost, and is of appropriate docility and size for laboratory procedures (Luginbuhl et al. 1974).

Swine develop atherosclerosis spontaneously with age and the distibution of the lesions in the aorta as well as the coronary arteries is similar to that seen in man. Slaughterhouse surveys (Gottlieb and Lalick 1954) and examination of pigs used in long term experiments (Skold and Getty 1961) have demonstrated that the pig develops atherosclerotic plaques in the heart and blood vessels similar to those observed in humans. Ratcliffe et al. (1970) reported lesions of atherosclerosis in swine of three different age groups to be morphologically identical to the corresponding stages of atherosclerosis in humans and, concluded that swine can replace nonhuman primates as subjects for studies of atherosclerosis. The circulatory system and heart of the pig resemble those of humans more closely than do those of most other mammals (Lumb and Singletay 1962; Guiney 1965). In addition, the lipoprotein pattern of swine is very similar to that found in humans (Calvert and Scott 1974; Mahley and Weisgraber 1974). A partial list of atherosclerotic studies performed with swine

includes the work of Bragdon et al. (1957), Cevallos et al. (1979), Downie et al. (1963), Greer et al. (1966), Gupta et al. (1974), Gyorkey and Reiser (1964), Lee et al. (1974), Link et al. (1972), Moreland et al. (1963), Nam et al. (1973) and Ratcliffe and Luginbuhl (1971).

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V. INFLUENCE OF GRADED LEVELS OF DIETARY PROPIONIC ACID ON PERFORMANCE AND CARCASS TRAITS OF SWINE FED DIETS SUPPLEMENTED WITH SOYBEAN MEAL OR CANOLA MEAL.[1]

A. Abstract

An experiment with sixty-four crossbred pigs averaging 24 kg liveweight at allotment was conducted to study the effects of dietary propionic acid (PA) on backfat thickness and fatty acid composition and on rate of gain and efficiency of feed utilization. Isonitrogenous and isoenergetic diets were based on either barley-soybean meal or barley-canola meal and contained either 0, 3, 6 or 9% PA. Inclusion of PA depressed feed intake in a linear manner with daily gain being reduced when pigs received 6 or 9% PA. Efficiency of feed utilization did not differ significantly for PA treatments. Backfat thickness was significantly reduced by PA inclusion in the diet while carcass grade was slightly improved. Inclusion of PA significantly increased levels of 15:0, 17:0 and 17:1 fatty acids in the outer subcutaneous backfat layers with an accompanying decrease in 16:0 and 18:0 fatty acids. Higher levels of odd chain fatty acids which were observed in the backfat of PA-supplemented pigs suggest that not all the propionic acid is being

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[1] The material presented in chapter five of this thesis has been published in the December 1980 issue of the Canadian Journal of Animal Science :Thacker, P.A. and Bowland, J.P., 1980. Influence of graded levels of propionic acid on performance and carcass traits of swine fed diets supplemented with soybean meal or canola meal. Can. J. Anim. Sci. 60:971-978.

metabolized and that some is being directly incorporated into fatty acids. Pigs fed diets supplemented with 15.9 to 18.9% canola meal had reduced feed intake, lower daily gains, poorer feed conversion efficiencies and reduced total backfat compared with those fed diets supplemented with soybean meal. Pigs fed canola meal-supplemented diets had higher levels of 17:0 fatty acid in their backfat in comparison with those fed soybean meal. These results do not invalidate the current recommendation that up to 10% canola meal may be fed in diets of growing pigs.

B. Introduction

It is well established that in ruminants, the ingested feed is degraded in the rumen to short chain volatile fatty acids (VFA), primarily acetic, propionic, and butyric acid. These acids are readily absorbed from the digestive tract and supply approximately 70% of the metabolizable energy required by the ruminant (Seely et al. 1969).

Significant amounts of VFA have been shown to be absorbed from the large intestine of the pig (Imoto and Namioka 1978; Kass et al. 1980). The contributions of these VFA to the energy supply of the simple stomached animal is controversial. Estimates of the contribution of endogenously produced VFA to the maintenance energy requirements of swine vary from 5 to 28% (Friend et al. 1964; Farrell and Johnson 1972; Kass et al. 1980). Although the majority of the VFA produced is in the form of acetic acid, approximately 30% of

the fecal VFA was in the form of propionic acid (Friend et al. 1962).

Methylmalonyl-CoA, a breakdown product of propionic acid metabolism, has been shown to cause a reduction in fatty acid synthesis (Cardinale et al. 1970). The synthesis do novo of fatty acids from acetyl-CoA is catalyzed by two enzyme systems; acetyl-CoA carboxylase which catalyzes the formation of malonyl-CoA from acetyl-CoA, and fatty acid synthetase which catalyzes the synthesis of long chain fatty acids from malonyl-CoA. The activity of both of these enzymes has been shown to be markedly inhibited by the presence of methylmalonyl-CoA (Frenkel et al. 1973).

Bowland et al. (1971) fed swine a mixture of VFA or the sodium salt of these acids and studied their influence on performance and fatty acid composition of backfat. Their results indicated that pigs were able to efficiently utilize up to 8% of a mixture of volatile fatty acids with no significant changes in backfat composition. In further studies 4% propionic acid added to diets containing 10% unprocessed rapeseed improved $(P<0.01)$ daily gain of pigs with no significant influence on carcass measurements or backfat composition (Bowland 1972). With the present demand for leaner carcasses, an experiment was undertaken to study the effects of dietary propionic acid on backfat thickness and composition and to study its effects on rate of gain and on efficiency of feed utilization.

C. Materials and Methods

Animal Management

Sixty-four crossbred pigs (Lacombe x Yorkshire), weighing approximately 24 kg were assigned on the basis of sex, litter and weight to one of eight diets (Table V.1). Diets were based on either barley-soybean meal or barley-canola meal (rapeseed meal from low glucosinolate, low erucic acid rapeseed from Tower cultivar) and contained either 0, 3, 6 or 9% propionic acid. The experimental diets were formulated to be isonitrogenous and isoenergetic and to meet the recommended nutritional requirements of swine for the weight ranges studied.

Pigs were housed four to a pen (2 barrows and 2 gilts) in concrete floored pens measuring 1.5 x 3.9 m. Feed and water were supplied ad libitum. Pigs were weighed at weekly intervals and feed consumption was determined on a pen basis at the time of the weekly weighing. Individual pigs were slaughtered at an average weight of 92 kg at which time carcass weight was recorded and backfat thickness measured. These values were then utilized in calculating Canadian Carcass Value Indices (Anonymous 1968). Samples of the outer subcutaneous layer of backfat, from the area of the 5th or 6th rib were obtained from the chilled carcasses the day following slaughter using a hand operated coring device.

Lipid Analysis

Determination of the fatty acid composition of backfat was done following the method of Morrison and Smith (1964).

Approximately one gram of backfat was extracted with 20 mL of chloroform:methanol (2:1). The filtrate (0.5 mL) was then transferred to a 150 x 20 mm screw capped culture tube and the solvent evaporated under nitrogen using a heated water bath (50°C.)

Fatty acid methyl esters were prepared by heating the sample for 30 minutes in a boiling water bath with 15 mL of a reagent containing 35% boron trifluoride (10% boron trifluoride in methanol), 20% n-pentane and 45% methanol. The fatty acid methyl esters were extracted with 10 mL of pentane after addition of 5 mL of water, the tube shaken, and the pentane layer containing the fatty acid methyl esters removed and stored for analysis later.

Analysis of the fatty acid methyl esters was done in a Bendix 2500 gas chromatograph (Bendix Corporation, Process Instruments Division, Ronceverte, West Virginia) equipped with a four column oven, four flame ionization detectors and dual differential electrometers. Glass columns, 3.6 m long, with an internal diameter of 2 mm were packed with 6% diethylene glycol succinate on Chromosorb W-HP (80-100 mesh, Supelco Inc., Bellefonte, Pennsylvania). The column was operated at 190°C while injector and detector temperatures were maintained at 215°C.

Peak intergration values were obtained with an Autolab Minigrator (SpectraPhysics, Technical Marketing Associates, Mississauga, Ontario), and the peaks were reproduced on a Fisher Recordall Series 5000 (Fisher Scientific Co. Edmonton

Alberta). The identity of the fatty acid methyl esters was established by comparison ot the retention time of unknown peaks with those of standard compounds (Applied Science, P.O. Box 440 State College, Pennsylvania).

Statistical Analyses

Multivariate analyses of variance were computed using a University of Alberta computer program with sex, level of acid and type of protein supplement as the main effects. The effects of protein supplement and inclusion of acid were tested against pens in treatment while the effect of sex was tested against the sex by pens in treatment interaction. Average daily feed and feed efficiency were analyzed on a pen basis, as individual feed consumption was not recorded. Therefore no sex effects could be calculated for these two criteria. Where appropriate, means were compared using Student-Newman-Keuls multiple range test (Steel and Torrie 1960) preceded by a significant F-test (Waldo 1976). Analysis of covariance as outlined by Steel and Torrie (1960) was used to adjust certain carcass characteristics for differences in carcass weight.

D. Results and Discussion

Effects of Propionic Acid

The inclusion of propionic acid depressed feed intake in a linear manner with intake being depressed 8.4, 15.4 and 23.1% for the 3, 6, and 9% propionic acid treatments respectively (Table V.2). This reduction in feed intake is

not as great as the 12.4 and 27.0% reduction in the feed intake of rats fed diets containing 3 and 6% sodium propionate (Hogue and Elliot 1964).

The reduction in daily intake was reflected in lower average daily gains for pigs receiving the 6 and 9% propionic acid treatments while gains for pigs receiving the 3% propionic acid treatment were not significantly different from the control. A similar reduction in average daily gains has been reported for rats fed propionic acid (Hogue and Elliot 1964). Dryden and Hartman (1971) attribute the reduction in average daily gains when short odd chain fatty acids are fed to an increased requirement for vitamin B-12. The conversion of methylmalonyl-CoA to succinyl-CoA is catalyzed by methylmalonyl-CoA isomerase (E.C. 5.4, 99.2) an enzyme which utilizes vitamin B-12 as a coenzyme. These workers suggest that a mild vitamin B-12 deficiency could be induced in the rat by the addition of propionic acid to a diet which in itself does not cause a deficiency.

The inclusion of propionic acid in the diet appeared to improve feed efficiencies at all levels of inclusion, but the differences were not significant ($P>0.05$).

Pigs fed the 9% propionic acid treatment had significantly lower slaughter weights and carcass weights than did pigs from the other treatments (Table V.3). The inclusion of propionic acid significantly lowered backfat thickness at all levels of inclusion and as a result Carcass Value Index for these pigs was slightly improved. Because of

the differences in carcass weight between treatments, analysis of covariance was used to adjust backfat levels to a common weight; the results indicated the effects of propionic acid inclusion remained significant.

The effect of inclusion of propionic acid on the level of backfat can be explained largely on the basis of the reduced feed intake for the propionic acid treated pigs. Since their intake was significantly lower, it would be expected that they would have less energy available for fattening. However, it is possible that the reduction in backfat thickness may result from accumulation of methylmalonyl-CoA due to inadequate levels of vitamin B-12 for the conversion of methylmalonyl-CoA to succinyl-CoA.

Cardinale et al. (1970) carried out experiments to investigate the effects of methylmalonyl-CoA upon the incorporation of ^{14}C acetyl-CoA and 1, 3 ^{14}C malonyl-CoA into fatty acids. Their results showed that the addition of methylmalonyl-CoA to fatty acid synthesizing systems reduced the amount of 1,3 ^{14}C malonyl-CoA. incorporated into fatty acids.

Frenkel et al. (1973) studied the effects of methylmalonyl-CoA on acetyl-CoA carboxylase and fatty acid synthetase activity in rat liver. Substrate addition of methylmalonyl-CoA markedly inhibited the activity of both enzymes of fatty acid synthesis. Forward and Gompertz (1970) reported that methylmalonyl-CoA is a competitive inhibitor with respect to malonyl-CoA in both liver and brain

preparation.

Inclusion of propionic acid significantly increased the levels of the odd chain fatty acids 15:0, 17:0 and 17:1 in backfat (Table V.4). The increase in these fatty acids appeared to be at the expense of the saturated fatty acids 16:0 and 18:0.

These results would indicate that three carbon propionic acid through its intermediary propionyl-CoA is being used as a priming molecule for fat synthesis in place of the two carbon acetyl-CoA. Therefore, it would appear that not all of the propionic acid is being metabolized through succinyl-CoA and that some of the acid is being incorporated directly into fatty acids. Although methylmalonyl-CoA inhibits the cytoplasmic pathway of fatty acid synthesis, it has been shown to have no effect on the microsomal pathway of fatty acid elongation. (Forward and Gempertz 1970).

The lack of any significant quantities of branched chain fatty acids would indicate that methylmalonyl-CoA is itself not being incorporated into fatty acids. Frenkel et al. (1973) showed that methylmalonyl-CoA was a poor substrate for the fatty acid synthetase reaction. Garton et al. (1975) reported that in the liver of vitamin B-12 deficient baboons, less than 0.3% of the total fatty acids were branched chained.

Effects of Protein Supplement

Pigs fed diets supplemented with canola meal at levels of 15.9 to 18.9% of the diet had reduced feed intake, lower average daily gains and poorer feed conversion efficiencies compared with those pigs fed diets supplemented with soybean meal (Table V.2). McKinnon and Bowland (1977) reported similar results and attributed the reduction in performance to a depression in digestibility of energy and nitrogen when rapeseed meal completely replaces soybean meal as the protein supplement.

Use of canola meal as the protein supplement significantly reduced total backfat compared with pigs fed diets supplemented with soybean meal. A reduction in total backfat was observed by McKinnon and Bowland (1977) when a commercial rapeseed meal was used to replace soybean meal. Bowland and Newell (1974) observed a similar reduction when ground rapeseed from the low erucic acid cultivars Span and Zephyr were used as protein supplements.

Pigs fed diets containing canola meal had significantly higher levels of the odd chained fatty acid 17:0 and exhibited a trend towards increased levels of unsaturated fatty acids (Table V.4). Bowland and Newell (1974) reported that the addition of rapeseed meal from either Span or Zephyr cultivars of rapeseed to the diet decreased the levels of 16:0 and 18:0 and increased the levels of 18:2 and 18:3 in the backfat. The inclusion of canola meal in the diet did not result in any measurable amounts of erucic acid

(22:1) in backfat samples from treated pigs.

Effects of Sex

Throughout the twelve week feeding trial, the rate of gain of gilts and barrows was similar. Since pigs were not individually fed, it was not possible to determine the feed intakes and feed efficiencies for either sex.

Carcasses from barrows were observed to have significantly higher levels of backfat in comparison with gilts (8.24 cm vs 7.76 cm). The only difference in backfat composition attributable to sex was a greater level of 18:2 in gilts compared with barrows (7.12% vs 6.62%).

The overall results indicate that the inclusion of propionic acid in swine diets may reduce feed consumption with a concurrent improvement in feed efficiency and reduction in backfat thickness. Whether the reduction in backfat thickness is due to the reduced intake and slower growth rate of propionic acid treated pigs or due to the accumulation of methylmalonyl CoA and its effects on fat synthesis is not known. The significantly higher levels of the odd chain fatty acids observed in the backfat of propionic acid treated pigs suggest that not all the propionic acid is being metabolized and that some is being directly incorporated into fatty acids.

Use of canola meal (Tower cultivar) as the protein supplement at levels of 15.9 to 18.9% of the diet resulted in reduced intake, lower average daily gains and poorer feed conversion efficiency compared with those of pigs fed

soybean meal as the protein supplement.

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Table V.1 Composition of Diets.

Protein Source	Soybean Meal				Canola Meal			
Level of Propionic Acid (%)	0	3	6	9	0	3	6	9
Ingredients (%)								
Barley	79.39	76.38	73.56	70.62	77.02	72.80	70.06	67.22
Soybean Meal	11.75	12.50	13.10	13.80	-----	-----	-----	----
Canola meal	-----	-----	-----	-----	15.90	17.40	18.10	18.90
Tallow	5.38	4.52	3.60	2.66	4.58	4.30	3.34	2.38
Propionic Acid	----	3.00	6.00	9.00	----	3.00	6.00	9.00
Cellulose[1]	0.72	0.80	0.90	1.06	----	----	----	---
Dicalcium phosphate	0.26	0.30	0.34	0.36	----	----	----	---
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (iodized)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-Mineral Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient Analysis (as fed basis)								
Dry Matter (%)	87.0	87.7	86.2	86.2	88.2	87.8	87.1	86.3
Crude protein (%)	14.9	15.7	15.4	15.7	14.5	15.7	15.7	16.3
Acid detergent fiber (%)	6.8	8.0	7.4	7.1	8.6	10.1	10.6	10.6
Ash (%)	3.6	3.9	4.0	4.0	4.1	4.3	4.7	5.2
Ether extract (%)	4.8	5.1	4.1	4.1	5.2	5.9	4.8	3.6
Digestible energy (Mj/kg)	13.8	13.9	13.9	13.9	13.6	13.8	13.8	13.8

[1] Alfafloc, Lee Chemicals, 1119 Yonge Street, Toronto

[2] Supplied the following per kg of diet: 7500 I.U. vitamin A; 700 I.U. vitamin D; 22 I.U. vitamin E; 9.8 mg riboflavin; 69.2 mg calcium pantothenate; 64 mg niacin; 1616 mg choline chloride; 35.2 ug vitamin B-12; 48 mg manganese; 200 mg iron; 20.3 mg copper; 240 mg limestone; .1 mg selenium; 160 mg zinc; and 500 mg furizolidone.

Table V.2 Effects of propionic acid and protein source on swine performance.[1]

Criteria	Weeks	Level of Propionic Acid (%) 0	3	6	9	S.E.	Protein Source Soybean	Canola	S.E.
Average daily feed (kg)	0-3	1.80a	1.63b	1.45c	1.38c	0.04	1.61a	1.51b	0.03
	3-6	2.55	2.34	2.30	2.01	0.14	2.44	2.16	0.10
	6-9	3.07a	2.87a	2.51b	2.46b	0.10	2.77	2.68	0.07
	9-12	3.49a	3.15ab	2.96ab	2.56b	0.19	3.03	3.04	0.14
	Total	2.73a	2.50ab	2.31ab	2.10c	0.07	2.46	2.35	0.05
Average daily gain (kg)	0-3	0.59	0.56	0.52	0.49	0.03	0.59a	0.49b	0.02
	3-6	0.79a	0.77a	0.68b	0.69b	0.02	0.78a	0.69b	0.02
	6-9	0.80a	0.73b	0.65c	0.69bc	0.02	0.75a	0.68b	0.01
	9-12	0.79	0.80	0.74	0.65	0.04	0.78	0.71	0.02
	Total	0.74a	0.72a	0.65b	0.63b	0.02	0.72a	0.64b	0.01
Feed/gain (kg/kg)	0-3	3.05	2.91	2.79	2.85	0.20	2.73	3.08	0.14
	3-6	3.23	3.04	3.38	2.91	0.20	3.13	3.13	0.14
	6-9	3.84	3.98	3.86	3.56	0.14	3.69	3.94	0.10
	9-12	4.41	3.94	4.00	3.93	0.32	3.88	4.28	0.23
	Total	3.69	3.47	3.53	3.33	0.11	3.41	3.67	0.08

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[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Table V.3 Effects of propionic acid and protein source on swine carcass traits.[1]

	Level of Propionic Acid (%)					Protein Source		
Criteria	0	3	6	9	S.E.	Soybean	Canola	S.E.
Slaughter weight (kg)	95.0a	94.2a	92.2a	86.7b	2.1	95.1a	89.1b	1.5
Carcass weight (kg)	77.0a	76.1a	76.1a	69.5b	2.1	76.5a	72.9b	1.5
Dressing percentage (%)	80.7	80.8	82.5	77.9	1.5	79.1a	81.9b	1.1
Total backfat (cm)[2]	8.5a	8.3ab	8.0b	7.2c	0.1	8.2a	7.8b	0.1
Carcass value index[3]	100.0a	100.7a	101.7ab	102.1b	1.0	100.5	100.9	0.7

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

[2] Sum of two measurements (back and loin).

[3] Calculated from carcass weight and backfat measurement.

Table V.4 Effects of propionic acid and protein source on fatty acid composition of backfat.[1]

	Level of Propionic Acid (%)					Protein Source		
Fatty Acid[2]	0	3	6	9	S.E.	Soybean	Canola	S.E.
14:0	1.7	1.6	1.7	1.6	0.07	1.6	1.6	0.05
14:1	0.2	0.2	0.2	0.2	0.01	0.2	0.2	0.01
15:0	0.2a	0.2a	0.2a	0.3b	0.02	0.2	0.2	0.01
15:1	0.1	0.1	0.1	0.1	0.01	0.1	0.1	0.01
16:0	24.2	24.1	24.0	23.9	0.38	24.3	23.8	0.27
16:1	3.5	3.5	3.5	3.6	0.11	3.5	3.6	0.08
17:0	0.6a	0.7ab	0.8b	1.0c	0.04	0.7a	0.8b	0.03
17:1	0.7a	0.8a	0.9ab	1.2b	0.08	0.8	1.0	0.06
18:0	11.7a	11.3ab	10.7bc	10.5c	0.23	11.2	10.9	0.16
18:1	47.0	47.4	47.0	47.0	0.39	47.2	47.0	0.28
18:2	6.6	6.7	7.0	7.1	0.15	6.8	7.0	0.10
18:3	1.2	1.1	1.3	1.2	0.09	1.1	1.3	0.06
19:0	0.4	0.4	0.5	0.5	0.04	0.4	0.4	0.03
20:1	1.3	1.3	1.3	1.2	0.08	1.2	1.3	0.06
20:2	0.5	0.5	0.5	0.5	0.04	0.4	0.4	0.03
20:4	0.3	0.3	0.3	0.3	0.01	0.3	0.3	0.01
SFA[3]	38.7	37.8	37.4	37.1	0.41	38.3	37.9	0.29
UFA[4]	61.3	62.2	62.6	62.9	0.42	61.7	62.1	0.29

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

[2] Fatty acids designated by chain length and number of double bonds. [3] Sum of saturated fatty acids.

[4] Sum of unsaturated fatty acids.

VI. EFFECTS OF VITAMIN B-12 ON PERFORMANCE AND CARCASS TRAITS OF PIGS FED DIETS SUPPLEMENTED WITH PROPIONIC ACID OR CALCIUM PROPIONATE[2]

A. Abstract

Effects of vitamin B-12 supplementation of diets containing propionic acid (PA) or calcium propionate (CP) on average daily gain (ADG), feed conversion efficiency (FCE), backfat measurements and fatty acid (FA) composition, were studied utilizing eighty crossbred pigs averaging 22.5 kg. Diets based on barley-soybean meal, containing 0, 3.5 or 7% PA or CP were fed with and without the addition of 4.95 mg vitamin B-12/ kg diet. The addition of PA depressed average daily feed intake, while CP had no effect. ADG was reduced when either 7% PA or CP was fed, but ADG was slightly improved when 3.5% PA or CP was fed. FCE was improved when 3.5 or 7% PA or 3.5% CP was fed but 7% CP depressed FCE. Vitamin B-12 supplementation slightly improved the ADG and FCE of pigs fed CP but had no effect on pigs fed PA. None of these differences was significant at the 5% level. Addition of CP significantly reduced the thickness of carcass backfat in treated pigs. Supplementation with vitamin B-12 eliminated this reduction. A similar though non-significant

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[2] The material presented in chapter six of this thesis has been published in the September 1981 issue of the Canadian Journal of Animal Science : Thacker, P.A. and Bowland, J.P. 1981. Effects of vitamin B-12 on performance and carcass traits of swine fed diets supplemented with propionic acid or calcium propionate. Can. J. Anim. Sci. 61:775-782.

trend was observed with PA. Pigs fed 7 % PA or CP had significantly higher levels of odd-chain FA 17:0 and 17:1 in carcass backfat. Addition of vitamin B-12 mollified this effect. In addition, vitamin B-12 supplementation of PA treated pigs significantly increased the levels of the unsaturated FA 18:2 and 18:3.

B. Introduction

Methylmalonyl-CoA, a breakdown product of propionic acid (PA) metabolism, has been reported to cause a reduction in in vitro fatty acid (FA) synthesis (Cardinale et al. 1970). The reduction in fat synthesis would appear to be the result of inhibition of the two key fat synthesizing enzymes, acetyl-CoA carboxylase and FA synthetase, by methylmalonyl-CoA (Frenkel et al. 1973).

Vitamin B-12 is a critical cofactor in the oxidative pathway of PA metabolism. It is essential for the metabolic conversion of methylmalonyl-CoA to succinyl-CoA catalyzed by methylmalonyl-CoA mutase (E.C. 5.4,99.2). When levels of vitamin B-12 are inadequate, methylmalonyl-CoA will accumulate in the tissues (Frenkel et al. 1974).

Recent work has shown that the inclusion of PA in swine diets significantly reduced the total backfat thickness of treated pigs (Thacker and Bowland 1980). It is not known whether the reduction in backfat thickness was due to a direct effect of PA on fat synthesis or simply a reflection of the reduced feed intake of treated pigs. Bowland et al.

(1971), reported improved palatability of diets containing the sodium salt of propionic acid compared with PA. Therefore, an experiment was undertaken to determine the effects of vitamin B-12 supplementation of diets containing PA or calcium propionate (CP) on backfat thickness and FA composition and to study its effects on rate of gain and efficiency of feed utilization.

C. Materials and Methods

Animal Management

Eighty crossbred pigs (Lacombe x Yorkshire) weighing approximately 22.5 kg were assigned on the basis of sex, litter and weight to one of ten dietary treatments (Table VI.1). Diets were based on barley-soybean meal and contained 0, 3.5 or 7% PA or CP fed with or without the addition of 4.95 mg vitamin B-12/ kg diet. The requirement for vitamin B-12 for 20-35 kg pigs receiving diets containing 3380 kcal digestible energy/ kg diet is reported to be 11 ug/ kg diet (NAS-NRC 1979). Due to lack of pen space, only the diet containing 7% CP was fed with and without vitamin B-12 while all diets containing PA were fed supplemented and unsupplemented with vitamin B-12.

All diets were formulated to meet the recommended nutrient requirements of swine for the weight ranges studied (NAS-NRC 1979). Diets containing CP were formulated to contain similar calcium:phosphorus ratios by manipulating the levels of dicalcium phosphate, limestone and

monoammonium phosphate (Table VI.1).

Pigs were housed 4 (2 barrows and 2 gilts) to a concrete-floored pen measuring 1.5 x 3.9 m. Feed and water were supplied ad libitum. Pigs were weighed at weekly intervals and feed consumption was determined on a pen basis at the same time. Pigs were slaughtered at an average weight of 90 kg at which time carcass weight was recorded and backfat measurements taken. These values were then utilized in calculating Canadian Carcass Value Indices (CVI). The CVI assigned to each carcass was the commercial grade, which is based on weight and backfat depth (shoulder and loin) determined according to the table of differentials in effect in 1980. Samples of the outer, subcutaneous layer of backfat from the area of the 5th or 6th rib were obtained from the chilled carcasses the day following slaughter.

Lipid Analysis

Details concerning lipid extraction and preparation of FA methyl esters have been described previously (Thacker and Bowland 1980). Analysis of the FA methyl esters was with a Varian Model 3700 Gas Chromatograph utilizing a Quadrex High Resolution glass capillary column coated with Silar-10C (Applied Science, P.O. Box 440, State College, Pennsylvania). The 10 m column had an internal diameter of 0.25 mm and an outside diameter of 0.75 mm. A linear temperature program was run from 140°C to 200°C at an increment of 4°C/ minute with nitrogen as the carrier gas.

Peak integration values were obtained with an Autolab minigrator (Spectra Physics, 2905 Stender Way, Santa Clara, California) and the peaks were reproduced on a Fisher Recordall Series 5000 (Fisher Scientific Co., Edmonton, Alberta). The identity of the FA methyl esters was established by comparison with the retention times of standard compounds (Applied Science, P.O. Box 440, State College, Pennsylvania). The unsaturated nature of some peaks was confirmed upon disappearance of the peak following bromination.

Statistical Analyses

A least squares analysis of variance was conducted using a University of Alberta computer program with sex, inclusion of acid or salt and vitamin B-12 supplementation as the main effects. All two-level interactions were identified and tested for significance. Average daily feed (ADF) intake and feed conversion efficiency (FCE) were analyzed on a pen basis (individual feed consumption was not recorded); therefore no sex effects could be calculated for these criteria.

The effects of propionate and vitamin supplementation and their interaction were tested against pens in treatment, while the effect of sex and its interactions were tested against the residual variation. Where appropriate, logical groupings of means were tested using Student-Newman-Keuls multiple range test as outlined by Winer (1962) when preceded by a significant F-test (Waldo 1976).

D. Results

Effects of Propionic Acid or Calcium Propionate

Dietary inclusion of 3.5 or 7.0 % PA depressed ADF 5.3 and 9.2 % in comparison with the control group (Table VI.2). Average daily gain (ADG) was reduced 4% in pigs fed 7% PA while those fed 3.5% PA gained at the same rate as the controls. Dietary inclusion of 3.5 or 7% PA resulted in a 6% improvement in FCE. None of these differences was statistically significant ($p>0.05$).

Dietary inclusion of CP did not depress ADF to the same extent as did PA. Pigs fed 3.5 % CP gained 4.5 % faster than the control while those fed 7% CP gained 8.3 % slower than the control. Addition of 3.5 % CP improved FCE 6 % while the addition of 7 % CP resulted in a 6 % decrease in FCE.

No significant differences were observed in slaughter weight, carcass weight, dressing percentage or CVI due to addition of PA or CP (Table VI.3). Addition of 7% CP resulted in a significant ($p<.05$) reduction in carcass backfat thickness. A similar though non-significant trend was observed for PA. Inclusion of PA and CP at the 7% level significantly ($p<0.05$) increased the levels of the odd chain FA 17:0 and 17:1 (Table VI.4).

Effects of Vitamin B-12

Supplementation of diets containing PA or CP with vitamin B-12 did not appear to have any effect on ADF (Table VI.2). Pigs fed CP containing diets supplemented with vitamin B-12 gained 8% faster than did unsupplemented

controls, while there was no effect on ADG due to supplementation of diets containing PA with vitamin B-12. Vitamin B-12 supplementation of diets containing CP improved FCE approximately 7 % while there was no change as a result of vitamin B-12 supplementation of diets containing PA.

There was no apparent effect of vitamin B-12 supplementation on slaughter weight, carcass weight, dressing percentage or CVI (Table VI.3). Pigs fed CP containing diets supplemented with vitamin B-12 had a 10.8 % increase ($p<0.05$) in carcass backfat thickness in comparison with those fed unsupplemented diets. There was no change in backfat thickness between supplemented and unsupplemented pigs fed PA. Supplementation of diets containing CP with vitamin B-12 resulted in significant decreases in the odd chain FA 17:0 and 17:1 while supplementation of diets containing PA with vitamin B-12 increased the levels of the unsaturated FA 18:2 and 18:3 ($p<0.05$) (Table VI.4).

Effects of Sex

During the ten week feeding trial castrated males grew significantly faster than females (.87 kg/day vs .81 kg/day, $p <0.10$) (Table VI.2). Males had significantly thicker carcass fat (11.6 cm vs 10.0 cm, $p<0.10$) (Table VI.3) and as a consequence had lower Carcass Value Indices (103.9 vs 104.4, $p<0.10$). There were no differences in backfat composition attributable to sex (Table VI.4).

E. Discussion

The results of the present experiment indicate that the reduction in carcass backfat thickness in calcium propionate treated pigs is not solely the result of a reduction in ADF. Pigs fed diets containing 7 % CP had essentially the same ADF yet had significantly less carcass backfat in comparison with the control. The depression in carcass backfat was removed by supplementation with vitamin B-12. The only known vitamin B-12 dependant reaction directly involved in lipid metabolism is the conversion of methylmalonyl-CoA to succinyl-CoA. In the absence of adequate levels of vitamin B-12 to make this conversion, methylmalonyl-CoA will accumulate in the body tissues (Frenkel et al. 1974).

Methylmalonyl-CoA has been shown to inhibit the activity of FA synthetase (Frenkel and Kitchens 1977) and acetyl-CoA carboxylase (Frenkel et al. 1973) and as a consequence FA synthesis has been shown to be reduced (Cardinale et al. 1970). It would appear that the mode of action of methylmalonyl-CoA is that of a competitive inhibitor with respect to malonyl-CoA (Forward and Gompertz 1970, Frenkel and Kitchens, 1977). Methylmalonyl-CoA binds covalently to the FA synthetase system (Forward and Gompertz 1970) but it is a poor substrate resulting in the formation of product at a low rate (Frenkel et al 1973).

Higher liver lipid levels have been observed in vitamin B-12 deficiency (Akesson et al. 1979) and in PA treated pigs (Thacker and Bowland 1981a). In addition, lower levels of

low density lipoproteins (LDL) have been observed in the serum of PA treated pigs (Thacker and Bowland 1981b). LDL are derived as a catabolic product of very low density lipoproteins (VLDL), the principle transport vehicle of triglyceride from the liver to extrahepatic tissues (Grundy 1978). Therefore, the reduced levels of carcass backfat in PA treated pigs may be the result of a reduction in lipid transport from liver to adipose tissue.

The increase in odd chain FA for PA and CP treated pigs was similar to that reported previously (Thacker and Bowland 1980). However, in previous work it appeared that the increase in odd chain fatty acids was at the expense of the saturated FA 16:0 and 18:0 while no specific changes were noted in these FA in the present work. Fatty acids are normally synthesized from acetyl-CoA and malonyl-CoA condensing on FA synthetase. The production of odd chain FA occurs when propionyl-CoA is substituted for acetyl-CoA. The lack of branched chain FA supports the hypothesis that methylmalonyl-CoA is not a good substrate for the FA synthetase reaction.

The overall results support previous findings that dietary propionate will reduce the backfat thickness of treated pigs (Thacker and Bowland 1980). The results suggest that the reduction in backfat thickness is not strictly due to a reduction in feed intake but may also be the result of the accumulation of methylmalonyl-CoA, a breakdown product of PA. Vitamin B-12 supplementation might prevent the

accumulation of methylmalonyl-CoA and eliminate its effects on fat synthesis.

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Table VI.1 Composition of Diets.

	Propionic Acid						Calcium Propionate			
Vitamin Supplement	Without B-12			With B-12			Without B-12			With B-12
Level of Propionate	0	3.5	7.0	0	3.5	7.0	0	3.5	7.0	7.0
Ingredients (%)										
Barley	77.30	73.25	69.20	76.55	72.50	68.45	69.60	67.20	64.90	64.15
Soybean Meal	14.00	14.90	15.80	14.00	14.90	15.80	15.90	16.40	16.90	16.90
Tallow	5.40	5.00	4.60	5.40	5.00	4.60	7.50	7.00	6.60	6.60
Propionic Acid	----	3.50	7.00	----	3.50	7.00	----	----	----	---
Calcium propionate	----	----	----	----	----	----	----	3.50	7.00	7.00
Dicalcium phosphate	0.70	0.75	0.80	0.70	0.75	0.80	4.00	2.00	----	---
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.50	0.80	----	---
Salt (iodized)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin B-12[2]	----	----	----	0.75	0.75	0.75	----	----	----	0.75
Monoammonium phosphate	----	----	----	----	----	----	----	1.60	3.10	3.10
Nutrient Analysis (as fed basis)										
Dry Matter (%)	89.3	89.9	89.5	90.8	89.6	90.2	90.0	90.4	91.0	90.8
Crude protein (%)	15.8	15.4	15.9	15.8	15.3	15.8	16.1	17.1	18.8	19.1
Acid detergent fiber (%)	6.7	7.0	5.7	6.6	6.8	6.4	6.4	5.2	5.6	5.3
Ash (%)	5.6	6.4	5.4	5.9	6.2	5.9	8.9	9.2	9.1	9.7
Calcium (%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ether extract (%)	7.4	7.5	6.7	7.8	6.5	7.8	9.3	7.4	7.0	7.7
Digestible energy (MJ/kg)[3]	13.7	13.9	14.1	13.7	13.9	14.1	13.7	14.0	14.1	14.1

[1] Supplied the following per kg of diet: 7500 I.U. vitamin A; 700 I.U. vitamin D; 22 I.U. vitamin E; 9.8 mg riboflavin; 69.2 mg calcium pantothenate; 64 mg niacin; 1616 mg choline chloride; 35.2 ug vitamin B-12; 48 mg manganese; 200 mg iron; 20.3 mg copper; 240 mg limestone; .1 mg selenium; 160 mg zinc; and 500 mg furizolidone.

[2] Contained 660 mg vitamin B-12 / kg supplement in a barley carrier.

[3] The D.E. values were calculated using bomb calorimeter values of 19.2 MJ/kg for propionic acid and 14.8 MJ/kg for calcium propionate assuming 90% digestibility.

Table VI.2 Effects of dietary vitamin B-12 on performance of swine fed propionic acid or calcium propionate.[1]

Criteria	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
Avg. daily feed (kg)	PA	2.26 ± .08	2.14 ± .08	2.05 ± .08	2.17 ± .07	2.12 ± .07[2]
	CP	2.35 ± .15	2.32 ± .15	2.28 ± .09	2.29 ± .07	2.33 ± .16
Avg. daily gain (kg)	PA	0.79 ± .03	0.80 ± .03	0.76 ± .03	0.79 ± .02	0.79 ± .02
	CP	0.84 ± .05	0.88 ± .05	0.77 ± .03	0.79 ± .02	0.86 ± .05
Feed / gain (kg/kg)	PA	2.85 ± .05	2.66 ± .05	2.69 ± .05	2.74 ± .04	2.68 ± .04
	CP	2.76 ± .11	2.59 ± .11	2.96 ± .06	2.90 ± .16	2.70 ± .12

[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different ($P<0.05$).

[2] Means ± S.E.M.

Table VI.3 Effects of dietary vitamin B-12 on carcass characteristics of swine fed propionic acid or calcium propionate.[1]

Criteria	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
Slaughter weight (kg)	PA	89.6 ± 1.7	89.7 ± 1.8	88.8 ± 1.8	89.3 ± 1.4	89.4 ± 1.5[4]
	CP	92.3 ± 2.3	91.1 ± 2.4	87.9 ± 1.4	89.4 ± 1.1	91.5 ± 2.3
Carcass weight (kg)	PA	72.4 ± 1.3	72.3 ± 1.4	71.2 ± 1.4	71.8 ± 1.1	72.1 ± 1.1
	CP	75.2 ± 1.7	73.7 ± 1.8	69.4 ± 1.0	71.0 ± 0.9	74.6 ± 1.9
Dressing percentage (%)	PA	80.8 ± 0.4	80.5 ± 0.4	80.3 ± 0.4	80.4 ± 0.3	80.7 ± 0.3
	CP	81.3 ± 0.8	80.8 ± 0.8	79.1 ± 0.5	79.4 ± 0.4	81.4 ± 0.8
Carcass backfat (cm)[2]	PA	11.4 ± 0.3	11.5 ± 0.3	11.1 ± 0.3	11.4 ± 0.2	11.2 ± 0.2
	CP	12.2 ± 0.5a	11.4 ± 0.5ab	10.4 ± 0.3b	10.7 ± 0.6a	12.0 ± 0.6b
Carcass value index[3]	PA	103.3 ± 0.5	102.4 ± 0.6	102.9 ± 0.6	102.7 ± 0.5	103.0 ± 0.4
	CP	102.3 ± 1.2	103.9 ± 1.3	103.4 ± 0.8	103.5 ± 0.6	102.9 ± 1.4

[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different ($P<0.05$).

[2] Sum of two measurements (back and loin).

[3] Calculated from carcass weight and backfat measurement.

[4] Means ± S.E.M.

Table VI.4. Effects of dietary vitamin B-12 on the fatty acid composition of backfat in pigs fed propionic acid or calcium propionate.[1]

Fatty Acid (% of total)	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
14:0	PA	1.3 ± .05	1.3 ± .04	1.3 ± .04	1.3 ± .04	1.3 ± .03
	CP	1.3 ± .04	1.3 ± .04	1.3 ± .02	1.3 ± .02	1.3 ± .04
15:0	PA	0.1 ± .01	0.1 ± .01	0.1 ± .01	0.1 ± .01	0.1 ± .01
	CP	0.1 ± .01	0.1 ± .01	0.1 ± .01	0.1 ± .01	0.1 ± .01
16:0	PA	22.1 ± .45	21.8 ± .50	22.0 ± .39	22.3 ± .38	21.5 ± .30
	CP	21.9 ± .46	21.0 ± .42	21.3 ± .24	21.3 ± .21	21.5 ± .43
16:1	PA	2.7 ± .08	2.9 ± .11	2.7 ± .08	2.8 ± .08	2.7 ± .06
	CP	2.5 ± .15	2.4 ± .13	3.0 ± .08	2.9 ± .06	2.5 ± .14
17:0	PA	0.4 ± .04a	0.5 ± .05a	0.7 ± .04b	0.6 ± .04	0.5 ± .03
	CP	0.4 ± .04a	0.4 ± .03a	0.6 ± .02b	0.5 ± .02a	0.4 ± .03b
17:1	PA	0.4 ± .02a	0.5 ± .03ab	0.6 ± .03b	0.5 ± .02a	0.7 ± .05b
	CP	0.5 ± .09a	0.5 ± .09ab	0.8 ± .06b	0.7 ± .05a	0.5 ± .03b
18:0	PA	10.8 ± .29	10.1 ± .41	10.6 ± .32	10.7 ± .31	10.3 ± .25
	CP	10.7 ± .59	10.9 ± .54	9.7 ± .30	9.9 ± .27	11.0 ± .56
18:1	PA	50.6 ± .39	50.9 ± .55	50.3 ± .42	50.7 ± .42	50.5 ± .33
	CP	50.3 ± .77	50.4 ± .71	51.6 ± .35	51.6 ± .35	49.9 ± .73
18:2	PA	8.5 ± .27	8.9 ± .39	8.9 ± .30	8.3 ± .30a	9.3 ± .23b
	CP	9.7 ± .32	10.2 ± .30	8.6 ± .17	9.2 ± .15	9.8 ± .31
18:3	PA	1.2 ± .07	1.0 ± .10	1.0 ± .08	0.9 ± .07a	1.2 ± .06b
	CP	1.1 ± .15	1.1 ± .14	1.2 ± .08	1.0 ± .07	1.2 ± .14
20:0	PA	0.8 ± .13	0.7 ± .19	0.8 ± .14	0.7 ± .14	0.8 ± .11
	CP	0.6 ± .22	0.6 ± .20	0.9 ± .11	0.8 ± .10	0.6 ± .21
20.2	PA	0.4 ± .06	0.3 ± .08	0.3 ± .07	0.3 ± .06	0.4 ± .05
	CP	0.4 ± .02	0.4 ± .02	0.3 ± .02	0.3 ± .01	0.4 ± .02
20:4	PA	0.3 ± .02	0.3 ± .02	0.3 ± .02	0.3 ± .02	0.4 ± .02
	CP	0.3 ± .02	0.3 ± .02	0.3 ± .02	0.3 ± .02	0.3 ± .02

[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different

VII. EFFECTS OF DIETARY PROPIONIC ACID ON SERUM LIPIDS AND LIPOPROTEINS OF PIGS FED DIETS SUPPLEMENTED WITH SOYBEAN MEAL OR CANOLA MEAL.[3]

A. Abstract

Sixty-four crossbred pigs averaging 24 kg liveweight at allotment were used to study the effects of dietary propionic acid (PA) on cholesterol metabolism. Isonitrogenous and isoenergetic diets were based on either barley-soybean meal or barley-canola meal and contained either 0, 3, 6 or 9% PA. Inclusion of PA significantly reduced the levels of total serum cholesterol, associated entirely with a reduction in the high density lipoprotein fraction. There was a slight increase in the lipid content of both hearts and livers due to PA inclusion; hearts showed slight elevations in triglycerides and livers had slightly elevated cholesterol and phospholipid levels. Pigs fed diets supplemented with soybean meal had lower levels of serum calcium, higher serum urea nitrogen, lower total serum cholesterol, lower low density lipoprotein cholesterol and lower circulating triglycerides compared with those fed canola meal. Hearts from pigs fed canola meal appeared to have higher levels of total lipid indicated by increases in

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[3] The material presented in chapter seven of this thesis has been published in the June 1981 issue of the Canadian Journal of Animal Science :Thacker, P.A., and Bowland, J.P., 1981. Effects of dietary propionic acid on serum lipids and lipoproteins of pigs fed diets supplemented with soybean meal or canola meal. Can. J. Anim. Sci. 61: 439-448.

triglycerides and phospholipids. There were no differences in lipid composition of livers due to source of protein supplement.

B. Introduction

Epidemiologic studies of the evolution of cardiovascular disease in human populations have emphasized the importance of an elevated serum total cholesterol as a risk factor in the development of coronary heart disease (Kannel et al. 1979; McGill 1979). However, recent data indicate that the concentration of high density lipoprotein (HDL) cholesterol is inversely related to the incidence of coronary heart disease (Grundy 1979; Havel 1979). It is hypothesized that this lipoprotein class participates in the removal of cholesterol from peripheral cells and delivery of cholesterol to the liver for excretion in the bile (Havel 1979). It is speculated that HDL may play a protective role in the prevention of cardiovascular disease (Grundy 1979).

The synthesis of cholesterol from acetyl-CoA is mediated by the cytoplasmic form of 3-hydroxy-3-methylglutaryl-CoA(HMG-CoA) synthase (Clinkenbeard et al. 1975). Bush and Milligan (1971) reported that the addition of propionate or propionyl-CoA to bovine liver homogenates inhibited the activity of HMG-CoA synthase.

Since the pig is considered to provide a suitable model for experimental atherosclerosis (Radcliffe and Luginbuhl

1971) and many similarities are found between pig and human lipoproteins and apolipoproteins (Fidge 1973), it was decided to study the effects of dietary inclusion of propionic acid on the levels of serum lipids and lipoproteins in the pig.

C. Materials and Methods

Animal Management

Sixty-four crossbred pigs (Lacombe x Yorkshire), weighing approximately 24 kg were assigned on the basis of sex, litter and weight to one of eight diets (Table VII.1). Diets were based on either barley-soybean meal or barley-canola meal (low glucosinolate and low erucic acid rapeseed meal from Tower cultivar) and contained either 0, 3, 6 or 9% propionic acid. The experimental diets were formulated to be isonitrogenous and isoenergetic and to meet the recommended nutritional requirements of swine for the weight ranges studied. An attempt was made to equalize fiber levels by the addition of cellulose (Alfafloc, Lee Chemicals, 1119 Yonge street,Toronto, Ontario) to the soybean meal supplemented diets.

Pigs were housed 4 (2 barrows and 2 gilts) to a concrete floored pen measuring 1.5 x 3.9 m. Feed and water were supplied ad libitum. Pigs were weighed weekly and feed consumption was determined on a pen basis at the time of the weighing. The effects of propionic acid inclusion on performance and carcass traits of the pigs have been

reported previously (Thacker and Bowland 1980).

At the start of, and after 6 and 12 weeks on experiment, blood samples were collected in Vacutainer tubes from all pigs by anterior vena cava puncture (Carle and Dewhirst 1942). Serum samples were frozen and stored at -20°C until required for analysis. Individual pigs were slaughtered at an average weight of 92 kg at which time hearts and livers were removed, frozen, and stored at -20°C until analysis.

Chemical Analyses

The following profile of serum constituents was determined by use of a Technicon SBM 12/60 autoanalyzer as described by Smythe et al. (1967): calcium (Ca), inorganic phosphorus (P), glucose, cholesterol, total protein, albumin, blood urea nitrogen (BUN), uric acid, bilirubin, alkaline phosphatase (Alk P), lactate dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT) (Dr. H.S. Hanson and Associates, Medical Laboratory, Edmonton).

High density lipoprotein (HDL) cholesterol was determined by a modified method of Lopes-Virella et al. (1977). Prior to the assay, 4.0 mL of sodium phosphotungstate solution (5.0 g of phosphotungstic acid and 0.8 g of sodium hydroxide made up to 100 mL with distilled water) and 1.0 mL of 2.5 M magnesium chloride solution were mixed and 50 uL of the combined reagent was added to a 0.5 mL serum sample diluted 1:1 with distilled water. The sample was mixed, then centrifuged at 1500 x g for 30 minutes at

2°C. The supernatant was transferred to a 12 x 75 mm tube and the cholesterol concentration determined on an Abbott Biochromatic Analyzer (Model AEA-100, Abbott Laboratories Ltd., 2213 Birch Street,Vancouver, B.C.) using an enzymatic cholesterol reagent (Allain et al. 1974). Sample dilution was found to be necessary in order to obtain a complete separation of the high density fraction from fractions in the precipitate. Agarose gel electrophoresis was performed on the supernatant to confirm that complete separation had occurred. Low density lipoproteins (LDL) were determined using the formulae of Friedwald et al. (1972). Serum triglycerides were determined using the manual procedure of Mendez et al. (1975).

The lipid content of heart and liver was determined by the method of Atkinson et al. (1972). A 2.0 g sample of freeze dried tissue was weighed in a 20 x 150 mm teflon lined, screw-cap tube, and 10 mL chloroform and 18.9 mL of a methanol:water mixture (10 mL methanol and 8.9 mL water) were added. The sample was homogenized on a polytron (Brinkmann Instruments, Rexdale, Ontario) for 30 seconds and then centrifuged at 1500 x g for approximately 4 minutes. A 5 mL sample of the chloroform layer was transferred into a weighed aluminum dish using a calibrated syringe, the chloroform evaporated, the dish reweighed and the lipid content calculated. The remaining chloroform layer was kept for the determination of lipid classes.

Heart and liver lipids were separated into their respective lipid classes following the method of Sipos and Ackman (1978). A 2.0 uL sample of the lipid extract was spotted on Chromosorb-S rods (.9 mm x 150 mm quartz rods with a coating of silica gel) and developed in a solvent mixture of methylene chloride, chloroform, acetic acid and methanol (97:8:0.5:0.15) for 30 minutes. The rods were air dried for 5 minutes and transferred to the scanning frame of an Iatroscan TH-10 Mark 11 Analyzer (Technical Marketing Associates, 1661 Finfar Court, Mississauga, Ontario). The flame ionization detector was operated with a hydrogen gas flow of 160 mL/minute from a pressure of 0.75 kg/cm^2 and air was supplied at a flow rate of 2000 mL/minute. The analog signal from the detector produced a chromatogram and the height of the integral curve gave the area under each peak.

Statistical Analysis

Multivariate analyses of variance were computed with sex, level of acid and type of protein supplement as the main effects. The effects of protein supplement and inclusion of acid and their interaction were tested against pens in treatment, while the effect of sex was and its interactions were tested against the residual variation. Means were compared using Student-Newman-Keuls multiple range test (Steel and Torrie 1960) when preceded by a significant F-test (Waldo 1976).

D. Results

Effects of Propionic Acid

Inclusion of propionic acid in the diet did not have any significant effects on the concentrations of non-lipid serum components. Since no significant treatment differences were obtained between blood samples taken at 0, 6, and 12 weeks only the twelve week data are reported (Table VII.2). Initial and six week data are presented in the appendix. The absence of a significant effect indicated that dietary inclusion of propionic acid had no adverse effects on the general well-being of treated pigs. The levels of serum constituents are similar to those reported previously for pigs of this weight range (Bowland 1975).

Dietary inclusion of propionic acid significantly reduced the levels of total serum cholesterol of pigs fed 6 or 9% propionic acid (Table VII.3). Total serum cholesterol was 14% lower in these pigs compared with the control group. The reduction in total cholesterol appeared to be at the expense of the HDL fraction which was reduced 27% in these treatment groups. In contrast the concentrations of the LDL cholesterol were not affected by the inclusion of propionic acid. Total serum triglycerides were 26% lower on the 6 and 9% propionic acid treatments ($p<0.10$)

Inclusion of propionic acid in the diet did not significantly affect the lipid composition of hearts from treated pigs (Table VII.4). However, pigs fed the 9% propionic acid diet had 11% more lipid which appeared to be

the result of an increase in triglycerides of a similar magnitude. In addition, this treatment group had 40% less cholesterol esters compared with pigs fed the control diet.

Livers from propionic acid treated pigs had 12% more lipid which was reflected in increases in both the cholesterol (7-13% elevation) and phospholipid (15-21% elevation) fractions but none of these differences were significant at the 5% level (Table VII.4).

Effects of Protein Source

Animals fed diets supplemented with soybean meal exhibited significantly lower levels of circulating calcium at week 12 in comparison with those animals fed diets supplemented with canola meal (Table VII.2). Blood urea nitrogen levels were observed to be significantly higher on the soybean meal supplemented diets. No differences were observed in other non-lipid serum components as a result of differences in source of protein supplementation.

Pigs fed diets supplemented with soybean meal exhibited significantly lower levels of total serum cholesterol than did those fed diets supplemented with canola meal (Table VII.3). The reduction in total cholesterol was reflected in a lower LDL fraction with the HDL being virtually unaffected by differences in protein supplementation. Circulating levels of serum triglycerides were also significantly lower in the soybean meal supplemented group than the canola meal group.

Hearts from pigs fed canola meal had higher levels of total lipid which was reflected in increases in the triglyceride and phospholipid fraction but none of these differences were significant at the 5% level (Table VII.4). There were no observable differences in the lipid composition of livers due to source of protein (Table VII.5).

Effects of Sex

The only significant change in blood serum constituents due to sex was an increased level of BUN in barrows (13.2 vs 11.2 mg/100 mL). There were no differences between barrows and gilts in the lipid composition of either hearts or livers.

E. Discussion

Effects of Propionic Acid

The data reported here indicate that dietary inclusion of propionic acid will result in a reduction in total serum cholesterol. The synthesis of cholesterol from acetyl-CoA is mediated by the cytoplasmic form of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (Clinkenbeard et al. 1975). Bush and Milligan (1971) utilizing bovine liver incubations reported that propionic acid at concentrations of 30 and 15 mM inhibited HMG-CoA synthase activity by 58 and 30% respectively while propionyl-CoA at the same concentration as acetyl-CoA (0.5mM) reduced the activity of the enzyme by 46%. It has

been suggested that propionyl-CoA is a competitive inhibitor with respect to acetyl-CoA for the active site on the HMG-CoA synthase enzyme molecule (Middleton 1967).

The activity of HMG-CoA reductase, the rate limiting enzyme of cholesterol synthesis in animal tissues (Rodwell et al. 1976) may also be affected by propionic acid. Ide et al. (1978) studied the effects of various dietary fats on the activity of HMG-CoA reductase in rat liver microsomes. Their work indicated that inclusion of fats with shorter chain fatty acids resulted in lower enzyme activities whereas inclusion of longer chain fatty acids resulted in higher enzyme activities. Their work also indicated that one or more of the enzymes converting mevalonate to cholesterol may be regulated by dietary fat.

Hepatic synthesis of cholesterol is dependent to some extent on feed intake. A restriction in dietary calories has been shown to cause a reduction in hepatic synthesis of cholesterol which may be reflected in a decrease in serum cholesterol (Grundy, 1978). Therefore the reduction in serum cholesterol may be due to the reduced feed intake observed when propionic acid is fed (Thacker and Bowland 1980).

The reduction in total serum cholesterol observed in the present experiment appeared to be due entirely to a reduction in the HDL fraction as LDL cholesterol levels were not affected by treatment. HDL are secreted by both the liver and the gut and have phospholipids and small amounts of free cholesterol as obligate structural components. The

higher levels of phospholipids and cholesterol observed in the livers of propionic acid-treated pigs may therefore be indicative of a reduced hepatic synthesis of high density lipoprotein.

Type of dietary fat has been shown to affect the structure of plasma lipoproteins (Goh and Heimberg 1977). The preferential incorporation of unsaturated fatty acids into phospholipids has been postulated to result in the formation of lipoproteins with a faster turnover rate (Mead and Fillerup 1957). The feeding of propionic acid resulted in the production of odd chain fatty acids in carcass fat (Thacker and Bowland 1980) and it is possible that high density lipoproteins with these fatty acids incorporated in their structure may also have an altered rate of catabolism.

Effects of Protein Supplementation

The results reported here on the effect of soybean meal on serum cholesterol levels are consistent with reports of other investigators who have reported a substantial reduction in serum cholesterol associated with soybean protein feeding (Kim et al. 1978; Howard et al. 1965).

The mechanism by which dietary protein affects the level of serum cholesterol is as yet unknown. However, it has been reported that oxidation and turnover of cholesterol are slower in rabbits fed a cholesterol-free purified diet containing casein than in rabbits fed the same diet with soy protein isolate as the dietary protein (Huff and Carroll, 1977).

Howard et al. (1965) postulated that soybean meal contains a protective factor which prevented hypercholesterolemia in rabbits. Topping et al. (1970) have shown that dietary saponins found at a level of 0.6% in soybean meal (Gestetner et al. 1966) increase bile acid and neutral sterol excretion in the pig, which may reduce circulating cholesterol levels.

It is difficult to state what the clinical implications of the current findings are. A reduction in circulating cholesterol levels would appear to be beneficial, but the effects of a reduction at the expense of the HDL cholesterol fraction, hypothesized to exhibit a protective effect on atherosclerosis are uncertain (Smith 1980). It is interesting to note that the feeding of polyunsaturated fatty acids, long thought to lower the risk of atherosclerosis also reduces the total cholesterol at the expense of the HDL fraction (Shepard et al. 1978).

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Table VII.1 Composition of Diets.

Protein Source	Soybean Meal				Canola Meal			
Level of Propionic Acid (%)	0	3	6	9	0	3	6	9
Ingredients (%)								
Barley	79.39	76.38	73.56	70.62	77.02	72.80	70.06	67.22
Soybean Meal	11.75	12.50	13.10	13.80	-----	-----	-----	----
Canola meal	-----	-----	-----	-----	15.90	17.40	18.10	18.90
Tallow	5.38	4.52	3.60	2.66	4.58	4.30	3.34	2.38
Propionic Acid	----	3.00	6.00	9.00	----	3.00	6.00	9.00
Cellulose[1]	0.72	0.80	0.90	1.06	----	----	----	---
Dicalcium phosphate	0.26	0.30	0.34	0.36	----	----	----	---
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (iodized)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-Mineral Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient Analysis (as fed basis)								
Dry Matter (%)	87.0	87.7	86.2	86.2	88.2	87.8	87.1	86.3
Crude protein (%)	14.9	15.7	15.4	15.7	14.5	15.7	15.7	16.3
Acid detergent fiber (%)	6.8	8.0	7.4	7.1	8.6	10.1	10.6	10.6
Ash (%)	3.6	3.9	4.0	4.0	4.1	4.3	4.7	5.2
Ether extract (%)	4.8	5.1	4.1	4.1	5.2	5.9	4.8	3.6
Digestible energy (Mj/kg)	13.8	13.9	13.9	13.9	13.6	13.8	13.8	13.8

[1] Alfafloc, Lee Chemicals, 1119 Yonge Street, Toronto

[2] Supplied the following per kg of diet: 7500 I.U. vitamin A; 700 I.U. vitamin D; 22 I.U. vitamin E; 9.8 mg riboflavin; 69.2 mg calcium pantothenate; 64 mg niacin; 1616 mg choline chloride; 35.2 ug vitamin B-12; 48 mg manganese; 200 mg iron; 20.3 mg copper; 240 mg limestone; .1 mg selenium; 160 mg zinc; and 500 mg furizolidone.

Table VII.2 Effects of propionic acid and protein source on serum constituents of growing swine (12 Weeks).[1]

	Level of Propionic Acid (%)					Protein Source		
Criteria	0	3	6	9	S.E.	Soybean	Canola	S.E.
Calcium (mg/100 mL)	11.1	10.7	10.7	10.6	0.1	10.5a	11.1b	0.1
Phosphorus (mg/100 mL)	7.6	7.4	6.9	6.8	0.2	7.4	7.0	0.1
Glucose (mg/100 mL)	91	93	85	83	4.9	85	91	3.5
Blood urea nitrogen (mg/100 mL)	13.2	13.1	12.2	10.6	0.7	13.2a	11.3b	0.5
Uric acid (mg/100 mL)	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1
Total protein (g/100 mL)	6.8	6.9	6.7	6.8	0.1	6.7	6.9	0.1
Albumin (g/100 mL)	4.0	4.0	3.9	3.9	0.1	4.0	3.9	0.1
Bilirubin (mg/100 mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glutamate Oxaloacetic Transaminase (mU/mL)	63	85	66	48	8.3	58	74	5.9
Alkaline Phosphatase (mU/mL)	204	223	201	177	14.1	187	216	10.0
Lactic Dehydrogenase (mU/mL)	637	804	710	587	100	673	697	71

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Table VII.3 Effects of propionic acid and protein source on serum lipids and lipoproteins of growing swine (12 Weeks).[1]

	Level of Propionic Acid (%)					Protein Source		
Criteria	0	3	6	9	S.E.	Soybean	Canola	S.E.
Total cholesterol (mg/100 ml)	147.1a	146.9a	127.1b	126.1b	5.0	130.5a	143.2b	3.5
HDL cholesterol (mg/100 ml)	59.5a	56.3a	44.9b	43.5b	1.5	50.3	51.8	1.0
LDL cholesterol (mg/100 ml)	75.7	78.5	73.6	74.4	5.1	71.4	79.8	3.5
Triglycerides (mg/100 ml)	59.5	60.3	43.8	41.1	5.8	44.2a	58.1b	4.1

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Table VII.4 Effects of propionic acid and protein source on heart lipid composition.

	Level of Propionic Acid (%)					Protein Source		
Criteria	0	3	6	9	S.E.	Soybean	Canola	S.E.
Weight (g)	297	309	299	334	25.7	301	318	18.2
Total lipid (mg/100 g)	4240	3883	4226	4726	220	4068	4470	155
Cholesterol esters (mg/100 g)	10	8	9	6	1.2	8	8	0.8
Triglycerides (mg/100 g)	3332	3042	3341	3897	216	3242	3564	153
Cholesterol (mg/100 g)	54	48	54	50	2.8	50	53	2.0
Phospholipids (mg/100 g)	684	605	688	600	42.0	606	683	29.7

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Table VII.5 Effects of propionic acid and protein source on liver lipid composition.

Criteria	Level of Propionic Acid (%)					Protein Source		
	0	3	6	9	S.E.	Soybean	Canola	S.E.
Weight (g)	1995	2040	2062	1910	66.7	1981	2023	47.2
Total lipid (mg/100 g)	3183	3619	3482	3643	140.0	3547	3416	98.0
Cholesterol esters (mg/100 g)	46	41	42	43	1.9	44	42	1.4
Triglycerides (mg/100 g)	137	207	100	155	31.3	140	159	22.0
Cholesterol (mg/100 g)	150	162	165	172	8.1	165	159	5.7
Phospholipids (mg/100 g)	2056	2414	2514	2589	125.2	2416	2388	88.0

[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

VIII. EFFECTS OF VITAMIN B-12 ON SERUM LIPIDS AND LIPOPROTEINS OF PIGS FED DIETS SUPPLEMENTED WITH PROPIONIC ACID OR CALCIUM PROPIONATE.[4]

A. Abstract

Effects of vitamin B-12 supplementation of diets containing propionic acid (PA) or calcium propionate (CP) on feed intake, serum lipids and lipoproteins were studied utilizing eighty crossbred pigs averaging 22.5 kg. Diets, based on barley-soybean meal, containing 0, 3.5 or 7% PA or CP were fed with and without the addition of 4.95 mg vitamin B-12/kg diet. Inclusion of PA or CP with or without the addition of vitamin B-12 had no significant effect on feed intake. Addition of PA or CP depressed serum cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and serum triglycerides. Vitamin B-12 supplementation had no effect on serum total cholesterol or HDL cholesterol, caused a reduction in LDL cholesterol and tended to overcome the reduction in serum triglyceride. Supplementation of diets with vitamin B-12 reduced liver lipid levels largely as a result of a decrease in cholesterol and phospholipids. These data suggest that the cholesterol lowering effect of PA is not simply a result

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[4] The material presented in chapter eight of this thesis has been accepted for publication in the Canadian Journal of Animal Science :Thacker, P.A., Bowland, J.P. and Fenton, M., 1982. Effects of vitamin B-12 on serum lipids and lipoproteins of pigs fed diets supplemented with propionic acid or calcium propionate.

of a depression in feed intake but arises from a direct action of PA on cholesterol metabolism.

B. Introduction

Epidemiological and nutritional studies indicate that an elevation of plasma lipids, especially cholesterol, is one of the most important factors leading to the development of coronary heart disease (Kannel et al. 1979; McGill 1979). Recent work has shown that inclusion of propionic acid (PA) in swine diets resulted in a significant reduction in the total serum cholesterol levels of treated pigs (Thacker and Bowland 1981a). The mechanism leading to this reduction has not been fully elucidated.

The reduction in total cholesterol may be the result of inhibition of two cholesterogenic enzymes, 3-hydroxy-3-methylglutaryl (HMG-CoA) synthase and HMG-CoA reductase by PA (Bush and Milligan 1971, Ide et al. 1978). However, a restriction in dietary energy has been shown to cause a reduction in hepatic synthesis of cholesterol which may be reflected in a decrease in serum cholesterol (Grundy 1978). It is therefore uncertain whether the reduction in serum cholesterol observed in PA treated pigs is the result of a direct effect of PA on cholesterol metabolism or simply a reflection of the reduced feed intake of PA treated pigs (Thacker and Bowland 1980).

Feeding salts of PA does not depress feed intake to the same extent as does the feeding of PA (Bowland et al. 1971).

Therefore it was felt that feeding calcium propionate (CP) might determine if the reduction in serum cholesterol observed when propionate is fed, was the result of a direct effect of the propionate or simply a reflection of a caloric restriction resulting in fewer acetyl-CoA units being available for cholesterol synthesis.

Propionate is metabolized in mammalian tissues over a pathway involving its successive conversion to propionyl-CoA, methylmalonyl-CoA and succinyl-CoA. The last step in this conversion requires vitamin B-12 as a coenzyme and when levels of vitamin B-12 are inadequate, propionate may not be fully metabolized, leading to its accumulation in the body tissues (Frenkel et al. 1974). Therefore a trial was set up to determine the effects of vitamin B-12 supplementation of diets containing PA or CP on the serum lipid and lipoprotein composition of the growing pig.

C. Materials and Methods

Animal Management

Eighty crossbred pigs (Lacombe x Yorkshire) weighing approximately 22.5 kg were assigned, on the basis of sex, litter and weight to one of ten experimental diets (Table VIII.1). Diets were based on barley-soybean meal and contained 0, 3.5 or 7% PA or CP fed with or without the addition of 4.95 mg vitamin B-12/kg diet. The requirement for vitamin B-12 for 20-35 kg pigs receiving diets containing 3380 kcal digestible energy/kg diet is reported

to be 11 ug/kg diet (NAS-NRC 1979). The high level of supplementation was chosen to ensure adequate levels of vitamin B-12 to metabolize the high levels of propionate fed. Due to lack of pen space, only the diet containing 7% CP was supplemented with vitamin B-12 while all diets containing PA were fed supplemented and unsupplemented with vitamin B-12.

The experimental diets were formulated to meet the recommended nutrient requirements of swine for the weight ranges studied (NAS-NRC 1979). Diets containing CP were formulated to contain similar calcium/phosphorus ratios by manipulating the levels of dicalcium phosphate, limestone and monoammonium phosphate.

Pigs were housed 4 (2 barrows and 2 gilts) to a concrete-floored pen measuring 1.5 x 3.9 m. Feed and water were supplied ad libitum. Pigs were weighed at weekly intervals and feed consumption was determined on a pen basis at the time of the weighing.

At the start of, and after 5 and 10 weeks on experiment, blood samples were collected in Vacutainer tubes from all pigs by anterior vena cava puncture (Carle and Dewhirst 1942). Serum samples were frozen and stored at -20°C until required for analyses. Individual pigs were slaughtered at an average weight of 90 ± 1.8 kg at which time hearts and livers were removed, frozen, and stored at -20°C until analyzed .

Chemical Analysis

Total serum cholesterol was determined on a Technicon Autoanalyzer 11 (Technicon Instruments Corporation, Tarrytown, New York) utilizing an enzymatic cholesterol reagent (Allain et al. 1974).

High density lipoprotein (HDL) cholesterol was determined by a modified method of Lopes-Virella et al. (1977). Prior to the assay, 4.0 mL of sodium phosphotungstate solution (5.0 g of phosphotungstic acid and 0.8 g of sodium hydroxide made up to 100 mL with distilled water) and 1.0 mL of 2.5 M magnesium chloride solution were mixed, and 50 uL was added to a 0.5 mL serum sample diluted 1:1 with distilled water. The sample was mixed, then centrifuged at 1500 x g for 30 minutes at 2°C. The supernatant was transferred to a 12 x 75 mm tube and the cholesterol concentration determined on an Abbott Biochromatic Analyzer (Model AEA-100, Abbott Laboratories Ltd., 2213 Birch Street,Vancouver, B.C.).

Low density lipoprotein (LDL) cholesterol was determined using the formulae of Friedwald et al. (1972). Serum triglycerides were determined using the manual procedure of Mendez et al. (1975).

The lipid content of heart and liver was determined by the method of Atkinson et al. (1972). A 2.0 g sample of freeze dried tissue was weighed in a 20 x 150 mm teflon lined screw cap tube and 10 mL chloroform and 18.9 mL of a methanol:water mixture (10 mL methanol and 8.9 mL water)

were added. The sample was homogenized in a polytron (Brinkmann Instruments, Rexdale, Ontario) for 30 seconds and then centrifuged at 1500 x g for approximately 4 minutes. A 5 mL sample of the chloroform layer was transferred into a weighed aluminum dish using a calibrated syringe, the chloroform evaporated, the dish reweighed and the lipid content calculated. The remaining chloroform layer was kept for the determination of lipid classes.

Heart and liver lipids were separated into their respective lipid classes following the method of Sipos and Ackman (1978). A 2.0 uL sample of the lipid extract was spotted on Chromosorb-S rods (.9 mm x 150 mm quartz rods with a coating of silica gel) and developed in a solvent mixture of methylene chloride, chloroform, acetic acid and methanol (97:8:0.5:0.15) for 30 minutes. The rods were air dried for 5 minutes and transferred to the scanning frame of an Iatroscan TH-10 Mark 11 Analyzer (Technical Marketing Associates, 1661 Finfar Court, Mississauga, Ontario). The flame ionization detector was operated with a hydrogen gas flow of 160 mL/minute from a pressure of 0.75 kg/cm^2 and air was supplied at a flow rate of 2000 mL/minute. Peak integration values were obtained with an Autolab minigrator (Spectra Physics, 2905 Stender Way, Santa Clara, California) and the peaks were reproduced on a Fisher Recordall Series 5000 (Fisher Scientific Co., Edmonton, Alberta).

Statistical Analyses

A least squares analysis of variance was conducted using a University of Alberta computer program with sex, inclusion of acid or salt and vitamin B-12 supplementation as the main effects. All two-level interactions were identified and tested for significance.

The effects of propionate and vitamin B-12 supplementation and their interaction were tested against pens in treatment, while the effect of sex and its interactions were tested against the residual variation. Where appropriate, logical groupings of means were tested using Student-Newman-Keuls multiple range test as outlined by Winer (1962) when preceded by a significant F-test (Waldo 1976).

D. Results and Discussion

Feed Intake

Inclusion of PA or CP had no significant effect on the feed intake of treated pigs (Table VIII.2). However, there was a trend for intake to be decreased when pigs were fed PA while no depression in feed intake was observed in CP fed pigs. A significant reduction in intake was observed in previous studies when pigs were fed PA (Thacker and Bowland 1980). Vitamin B-12 supplementation had no apparent effect on the intake of pigs fed either PA or CP. The effects of PA, CP and vitamin B-12 supplementation on other performance traits has been reported previously (Thacker and Bowland

1981b).

Serum Cholesterol

Dietary inclusion of 3.5 and 7.0 % PA depressed total serum cholesterol 7.4 and 13.5 % ($P<0.05$) while inclusion of 3.5 and 7.0 % CP depressed serum cholesterol levels 20.2 and 16.9 % in comparison with the control ($P< 0.05$). The reduction in serum cholesterol in pigs fed PA is of a similar magnitude to that observed previously (Thacker and Bowland 1981a). Vitamin B-12 supplementation did not affect serum cholesterol levels in pigs fed either PA or CP.

Hepatic synthesis of cholesterol is dependent to some extent on feed intake. A restriction in dietary calories has been shown to cause a reduction in hepatic synthesis of cholesterol which may be reflected in a decrease in serum cholesterol (Grundy 1978). However it is evident from those pigs fed CP that the cholesterol lowering effect of propionate is not simply a reflection of a reduced feed intake but must be due to a direct effect of the propionate on cholesterol metabolism.

Propionic acid and other short chain fatty acids have been reported to affect the activity of the cholesterogenic enzymes HMG-CoA synthase and HMG-CoA reductase (Bush and Milligan 1971; Ide et al. 1978). A decrease in the activity of these key enzymes would affect the rate of cholesterol synthesis. Salomons (1977) reported a significant decrease in hepatic cholesterol synthesis in pigs fed PA.

High and Low Density Lipoproteins.

HDL cholesterol levels were reduced 9.6 and 11.7 % ($P<0.05$) in pigs fed 3.5 and 7.0 % PA while HDL cholesterol levels were reduced 11.1 and 24.1 % ($P<0.05$) in pigs fed 3.5 and 7.0 % CP (Table VIII.2). HDL levels were unaffected by supplementation with vitamin B-12.

It is hypothesized that the role of the HDL is to transport cholesterol from peripheral tissues to the liver for excretion in the bile (Havel 1979). Significantly elevated levels of cholesterol have been observed in adipose tissue of PA treated pigs (Thacker et al. 1981). The lower levels of HDL cholesterol observed in the present study suggest that one mechanism by which propionate reduces total serum cholesterol is through a shift in body cholesterol from serum to the tissue pool.

LDL cholesterol levels were reduced 11.9 and 13.1 % in pigs fed 3.5 and 7.0% PA while addition of 3.5 and 7.0 % CP reduced LDL cholesterol levels 28.5 ($P<0.05$) and 15.4 % in comparison with the control. Pigs fed PA containing diets supplemented with vitamin B-12 exhibited a 11.1 % ($P<0.05$) reduction in LDL cholesterol, while those fed CP containing diets supplemented with vitamin B-12 had a 13.1 % ($P<0.05$) reduction in LDL cholesterol. LDL are derived in the serum as catabolic products of very low density lipoproteins (VLDL), the principle transport vehicle of triglyceride from the liver to the extra-hepatic tissues (Grundy 1978).

Serum Triglycerides.

Although not statistically significant ($P<0.05$), there was a general trend for serum triglycerides to be reduced due to inclusion of either PA or CP (Table VIII.2). A similar trend was observed previously (Thacker and Bowland 1981a). The apparent reduction in serum triglycerides was overcome by supplementation with vitamin B-12. The only known vitamin B-12 dependent reaction directly involved in lipid metabolism is the conversion of methylmalonly-CoA to succinyl-CoA. Methylmalonyl-CoA, a breakdown product of PA metabolism, has been shown to inhibit the activity of acetyl-CoA carboxylase (Frenkel et al. 1973) and fatty acid synthetase (Frenkel and Kitchens 1977) and, as a consequence fat synthesis has been shown to be reduced (Cardinale et al. 1970). The observation of reduced LDL and serum triglyceride levels may explain the reduced backfat levels observed in PA-treated pigs (Thacker and Bowland 1980).

Liver and Heart Lipid Composition

Liver weight and liver lipid composition were not significantly affected by inclusion of either PA of CP (Table VIII.3). Pigs fed CP diets supplemented with vitamin B-12 had a 8.2% ($P<0.05$) reduction in liver lipid levels in comparison with unsupplemented animals. The reduction in total lipid appeared to be the result of a decrease in cholesterol and phospholipid. Pigs fed PA-containing diets supplemented with vitamin B-12 showed reductions of 12.5% ($P<0.05$) and 8.3 % ($P<0.05$) for cholesterol and phospholipid

respectively. CP-fed pigs showed reductions of 22.2 % ($P<0.05$) and 9.4 % ($P<0.05$) in cholesterol and phospholipid due to vitamin B-12 supplementation.

Heart weight and heart lipid composition were unaffected by PA, CP or vitamin B-12 supplementation (Table VIII.4).

The general lack of effect of vitamin B-12 on cholesterol metabolism may be explained by the fact that vitamin B-12 is only required in the conversion of methylmalonyl-CoA to succinyl-CoA and the remainder of the metabolic pathway for propionate catabolism is unaffected by this vitamin. The increase in serum triglycerides due to vitamin B-12 supplementation suggests that the inhibitory effects of methylmalonyl-CoA on fat metabolism are removed by supplementation with vitamin B-12.

The overall results support previous findings that dietary propionate will reduce total serum cholesterol (Thacker and Bowland 1981a). The data indicate that the reduction in serum cholesterol is not simply a reflection of a reduced feed intake but arises from a direct effect of PA on cholesterol metabolism. Lower levels of HDL cholesterol suggest that one mechanism by which PA reduces total serum cholesterol is through a shift in body cholesterol from serum to the tissue pool.

Although the results of this study are unlikely to have a direct influence on swine production, they suggest possible mechanisms for altering the lipid metabolism of

swine and provide information on the pig as a model in studies relating to human nutrition.

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Table VIII.1 Composition of Diets.

	Propionic Acid						Calcium Propionate			
	Without B-12			With B-12			Without B-12			With B-12
Ingredients (%)										
Barley	77.30	73.25	69.20	76.55	72.50	68.45	69.60	67.20	64.90	64.15
Soybean Meal	14.00	14.90	15.80	14.00	14.90	15.80	15.90	16.40	16.90	16.90
Tallow	5.40	5.00	4.60	5.40	5.00	4.60	7.50	7.00	6.60	6.60
Propionic Acid	----	3.50	7.00	----	3.50	7.00	----	3.50	7.00	7.00
Calcium propionate	----	----	----	----	----	----	----	3.50	7.00	7.00
Dicalcium phosphate	0.70	0.75	0.80	0.70	0.75	0.80	4.00	2.00	----	---
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Salt (iodized)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin B-12[2]	----	----	----	0.75	0.75	0.75	----	----	----	0.75
Monoammonium phosphate	----	----	----	----	----	----	----	1.60	3.10	3.10
Nutrient Analysis (as fed basis)										
Dry Matter (%)	89.3	89.9	89.5	90.8	89.6	90.2	90.0	90.4	91.0	90.8
Crude protein (%)	15.8	15.4	15.9	15.8	15.3	15.8	16.1	17.1	18.8	19.1
Acid detergent fiber (%)	6.7	7.0	5.7	6.6	6.8	6.4	6.4	5.2	5.6	5.3
Ash (%)	5.6	6.4	5.4	5.9	6.2	5.9	8.9	9.2	9.1	9.7
Calcium (%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ether extract (%)	7.4	7.5	6.7	7.8	6.5	7.8	9.3	7.4	7.0	7.7
Digestible energy (MJ/kg)[3]	13.7	13.9	14.1	13.7	13.9	14.1	13.7	14.0	14.1	14.1

[1] Supplied the following per kg of diet: 7500 I.U. vitamin A; 700 I.U. vitamin D; 22 I.U. vitamin E; 9.8 mg riboflavin; 69.2 mg calcium pantothenate; 64 mg niacin; 1616 mg choline chloride; 35.2 ug vitamin B-12; 48 mg manganese; 200 mg iron; 20.3 mg copper; 240 mg limestone; .1 mg selenium; 160 mg zinc; and 500 mg furizolidone.

[2] Contained 660 mg vitamin B-12 / kg supplement in a barley carrier.

[3] The D.E. values were arbitarily calculated using bomb calorimeter values of 19.2 MJ/kg for propionic acid and 14.8 MJ/kg for calcium propionate assuming 90% digestibility.

Table VIII.2 Effects of Propionic Acid, Calcium Propionate And Vitamin B-12 Supplementation on Feed Intake And Serum Lipids And Lipoproteins of Growing Swine (12 Weeks).[1]

Criteria	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
Avg. daily feed (kg)	PA	2.26 ± .08	2.14 ± .08	2.04 ± .08	2.12 ± .03	2.17 ± .03[2]
	CP	2.31 ± .12	2.28 ± .12	2.28 ± .08	2.28 ± .03	2.29 ± .03
Total cholesterol (mg / 100 ml)	PA	148 ± 3a	137 ± 3ab	128 ± 3b	141 ± 3	134 ± 3
	CP	153 ± 4a	122 ± 4b	127 ± 3b	138 ± 3	129 ± 5
HDL cholesterol (mg / 100 ml)	PA	51 ± 1a	46 ± 1ab	45 ± 1b	48 ± 1	47 ± 1
	CP	54 ± 1a	48 ± 1b	41 ± 1c	47 ± 1	48 ± 2
LDL cholesterol (mg / 100 ml)	PA	84 ± 3	74 ± 3	73 ± 4	81 ± 3a	72 ± 3b
	CP	84 ± 4a	60 ± 4b	71 ± 3ab	76 ± 3a	66 ± 5b
Triglycerides (mg / 100 ml)	PA	71 ± 11	78 ± 12	57 ± 10	61 ± 6	75 ± 6
	CP	80 ± 15	79 ± 15	69 ± 11	69 ± 6	83 ± 10

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[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different (P>0.05).

[2] Values are Means ± S.E.M.

Table VIII.3 Effects of Propionic Acid, Calcium Propionate and Vitamin B-12 Supplementation on Liver Lipid Composition.[1]

Criteria	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
Liver weight (kg)	PA	1645 ± 76	1778 ± 88	1798 ± 88	1753 ± 50	1726 ± 50 [2]
	CP	1648 ± 115	1713 ± 126	1863 ± 79	1753 ± 50	1753 ± 87
			mg / liver / 100 grams body weight			
Total lipid	PA	75.3 ± 2.1	80.6 ± 2.5	80.9 ± 2.4	81.4 ± 1.7	76.5 ± 1.7
	CP	76.3 ± 3.2	75.2 ± 3.5	79.6 ± 2.2	80.3 ± 1.7a	73.7 ± 3.0b
Cholesterol ester	PA	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2
	CP	1.7 ± 0.2	1.8 ± 0.3	2.0 ± 0.2	1.7 ± 0.2	1.9 ± 0.3
Triglyceride	PA	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	CP	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Free fatty acid	PA	6.7 ± 0.7	6.2 ± 0.8	5.5 ± 0.7	5.4 ± 0.5	6.8 ± 0.5
	CP	8.2 ± 1.0	6.1 ± 1.1	6.0 ± 0.7	6.6 ± 0.5	6.9 ± 0.9
Cholesterol	PA	2.7 ± 0.3	3.2 ± 0.3	3.1 ± 0.3	3.2 ± 0.1a	2.8 ± 0.1b
	CP	3.3 ± 0.4	2.9 ± 0.5	3.5 ± 0.3	3.6 ± 0.1a	2.8 ± 0.2b
Phospholipid	PA	63.5 ± 2.1	68.8 ± 2.4	69.9 ± 2.4	70.3 ± 3.2a	64.5 ± 3.2b
	CP	62.6 ± 3.2	64.0 ± 3.5	67.7 ± 2.2	67.9 ± 3.2a	61.5 ± 3.6b

[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different ($P>0.05$).

[2] Values are Means ± S.E.M.

Table VIII.4 Effects of Propionic Acid, Calcium Propionate and Vitamin B-12 Supplementation on Heart Lipid Composition.[1]

Criteria	Propionic Supplement	Level (%)			Vitamin B-12	
		0	3.5	7.0	-	+
Heart weight (kg)	PA	296 ± 6	314 ± 5	304 ± 5	300 ± 6	308 ± 6[2]
	CP	299 ± 6	335 ± 7	305 ± 6	311 ± 6	314 ± 10
		mg / heart / 100 grams body weight				
Total lipid	PA	29.2 ± 2.8	34.5 ± 2.2	32.6 ± 2.2	31.9 ± 3.6	32.2 ± 2.9
	CP	29.0 ± 3.0	32.6 ± 3.3	30.5 ± 2.0	31.5 ± 3.4	29.8 ± 3.0
Cholesterol ester	PA	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
	CP	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Triglyceride	PA	20.8 ± 3.0	27.8 ± 2.3	25.6 ± 2.4	24.6 ± 3.9	24.8 ± 3.2
	CP	21.6 ± 3.8	24.2 ± 3.5	22.6 ± 2.2	23.2 ± 3.2	23.3 ± 3.2
Free fatty acid	PA	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
	CP	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2
Cholesterol	PA	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
	CP	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Phospholipid	PA	6.9 ± 0.6	5.3 ± 0.5	5.8 ± 0.5	6.0 ± 0.8	5.9 ± 0.6
	CP	5.9 ± 0.7	6.9 ± 0.7	6.2 ± 0.4	6.6 ± 0.6	6.1 ± 0.8

[1] Within level of propionate or vitamin supplement, means with the same or no letter are not significantly different ($P>0.05$).

[2] Values are Means ± S.E.M.

IX. General Summary and Conclusions

The overall results presented in this thesis suggest that feeding propionic acid is effective in reducing the serum cholesterol levels of treated pigs. The observation of a reduction in the HDL fraction suggests that one mechanism by which propionate reduces total serum cholesterol is through a shift in body cholesterol from the serum to the tissue pool. The observation of an increase in carcass cholesterol levels supports this viewpoint (Thacker et al. 1981)[5] However, more work needs to be done to determine whether cholesterol synthesis or excretion is altered by propionate feeding.

It is difficult to state what the clinical implications of the current findings are. A reduction in circulating cholesterol levels would appear to be beneficial, but the effects of a reduction at the expense of the HDL fraction, hypothesized to exhibit a protective effect on atherosclerosis are uncertain. It is interesting to note that the feeding of polyunsaturated fatty acids, long thought to lower the risk of atherosclerosis also reduces the total cholesterol at the expense of the HDL fraction.

Although propionate is effective in reducing serum cholesterol, its clinical application may be limited due to the large doseage required to cause a reduction in serum

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[5] THACKER, P.A., SALOMONS, M.O., AHERNE, F.X., MILLIGAN, L.P. and BOWLAND, J.P. 1981. Influence of dietary propionic acid on the cholesterol metabolism of pigs fed hypercholesterolemic diets. Can. J. Anim. Sci. In Press, December 1981.

cholesterol and because of the poor palatability of propionate.

On a practical basis, it would appear that propionate is effective in reducing carcass backfat thickness in treated pigs. The reduction occurring when CP is fed suggests that the effects of propionate are not simply a reflection of reduced feed intake but arise from a direct effect of propionate on lipid metabolism. Vitamin B-12 supplement removed the inhibitory effects of propionate which suggests that the reduction in carcass backfat is due to the accumulation of methylmalonyl-CoA and its effects on fat synthesis. However, unless the price differential for lean carcasses is significantly increased, or the price of propionic acid appreciably reduced, the inclusion of propionate in swine rations cannot be recommended.

Propionic acid is a difficult compound to incorporate in feed mixes because of its odor and corrosive properties. These characteristics would also limit its use as a feed additive for swine.

Appendix 1. Effects of propionic acid and protein source on serum constituents of growing swine (Week 0)[1]

Criteria	Level of Propionic Acid (%)					Protein Source		
	0	3	6	9	S.E.	Soybean	Canola	S.E.
Calcium (mg/100 ml)	10.1	10.1	10.0	10.4	0.2	10.1	10.1	0.2
Phosphorus (mg/100 ml)	8.8	8.5	7.9	8.7	0.3	8.3	8.7	0.2
Glucose (mg/100 ml)	95	88	91	103	5.3	95	94	3.6
Blood urea nitrogen (mg/100 ml)	15.4	14.0	12.6	13.1	0.9	13.1	14.4	0.6
Uric acid (mg/100 ml)	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1
Total protein (g/100 ml)	5.9	6.0	5.8	6.0	0.1	6.0	5.9	0.1
Albumin (g/100 ml)	3.7	3.7	3.6	3.8	0.1	3.6	3.8	0.1
Bilirubin (mg/100 ml)	0.4	0.5	0.5	0.2	0.1	0.4	0.4	0.1
Glutamate Oxaloacetic Transaminase (mU/ml)	60	80	68	59	7.0	63	70	4.9
Alkaline Phosphatase (mU/ml)	100	124	184	218	26.2	176	137	18.3
Lactic Dehydrogenase (mU/ml)	197	99	279	307	81	205	236	56

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[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Appendix 2. Effects of propionic acid and protein source on serum constituents of growing swine (Week 6)[1]

	Level of Propionic Acid (%)					Protein Source		
Criteria	0	3	6	9	S.E.	Soybean	Canola	S.E.
Calcium (mg/100 ml)	9.2	9.6	9.0	9.3	0.7	9.6	8.9	0.5
Phosphorus (mg/100 ml)	6.6	7.7	6.5	6.0	0.5	7.2	6.2	0.3
Glucose (mg/100 ml)	93	93	94	98	6.6	97	92	4.6
Blood urea nitrogen (mg/100 ml)	9.3	10.2	10.0	8.8	0.9	10.6	8.5	0.7
Uric acid (mg/100 ml)	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1
Total protein (g/100 ml)	5.2	5.8	5.3	5.2	0.3	5.7	5.0	0.2
Albumin (g/100 ml)	2.8	3.1	2.9	3.0	0.2	3.2	2.7	0.2
Bilirubin (mg/100 ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glutamate Oxaloacetic Transmainase (mU/ml)	60	69	50	46	8.1	55	57	5.6
Alkaline Phosphatase (mU/ml)	209	179	240	262	20.0	218	227	14.1
Lactic Dehydrogenase (mU/ml)	340	550	448	379	89	438	421	62

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[1] Within level of acid or protein source, means with the same letter or no letter are not significantly different ($P<0.05$).

Appendix 3. Serum Constituents of Barrows and Gilts at Time 0 (Experiment 1)

Criteria	Barrows	Gilts	S.E.M.
Calcium (mg/100 ml)	10.2	10.1	0.15
Phosphorus (mg/100 ml)	8.7	8.3	0.16
Glucose (mg/100 ml)	95	93	2.3
Blood urea nitrogen (mg/100 ml)	14.4a	13.1b	0.3
Uric acid (mg/100 ml)	0.2	0.2	0.01
Total protein (mg/100 ml)	5.9	5.9	0.08
Albumin (mg/100 ml)	3.7	3.7	0.07
Bilirubin (mg/100 ml)	0.4	0.4	0.03
Glutamate Oxaloacetic Transaminase (mU/ mL)	69	65	4.3
Alkaline phosphatase (mU/ mL)	178	134	26
Lactic dehydrogenase (mU/ mL)	167	274	58

Appendix 4. Serum Constituents of Barrows and Gilts at Week 6 (Experiment 1)

Criteria	Barrows	Gilts	S.E.M.
Calcium (mg/100 ml)	9.3	9.2	0.30
Phosphorus (mg/100 ml)	6.7	6.7	0.20
Glucose (mg/100 ml)	97	91	2.9
Blood urea nitrogen (mg/100 ml)	10.4a	8.8b	0.3
Uric acid (mg/100 ml)	0.2	0.2	0.01
Total protein (mg/100 ml)	5.4	5.3	0.16
Albumin (mg/100 ml)	2.9	2.9	0.11
Bilirubin (mg/100 ml)	0.1	0.1	0.01
Glutamate Oxaloacetic Transaminase (mU/ mL)	54	58	2.7
Alkaline phosphatase (mU/ mL)	218	227	15
Lactic dehydrogenase (mU/ mL)	438	421	62

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